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SOME VARIATIONS IN THE CHELATING PROCESS FOR HISTOLOGIC SECTIONING OF HUMAN TEETH*

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The fundamental principle of using acid to remove mineral salts from bone and teeth has long been established. Descriptions of the use of various acids, all of which employ the same principle, can be found in most standard technic text books. In recent years, the passage of an electric current through the tissue when it is immersed in the various acid solutions has been used to hasten the decalcification process.[1] These methods have worked with varying degrees of excellence for bone and dentine, but the processing of enamel has been an unsolvable problem. Although there are various explanations for the phenomena, the fact remains, that when mature enamel has been subjected to acid, not only do the inorganic salts disappear, but also the supporting organic framework.[2] With the advent of chelating agents, there has come possibly a new attack on this old technic problem. Some procedures for using these chelating agents in an attempt to retain this organic framework in enamel is the subject for this report.

Literature

Using a saturated (10%) solution of disodium-dihydrogen versenate, Birge and Imhoff[3] decalcified thin slices of formalin-fixed bone until all the calcium had been removed, and the bone was soft and spongy. They did not wash the bone after decalcification, but dehydrated and processed it directly. The cells of the marrow were found to be well preserved, and the staining qualities were identical with bone marrow not subjected to decalcifying solution.

Hunter and Nikiforuk[4] tested the chelating solutions as to concentration, pH, temperature, time to decalcify, and effect of fixatives. They found that the stronger the solution, the shorter

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the time of decalcification; the rate of decalcification was retarded as the pH was increased; the higher temperature decreased the decalcification time, and that the chelating process was longer than the other decalcifying solutions except magnesium citrate at pH 7.5 They also varied the pH of the fixative solutions, and used zenker-formol solution as well as formalin solutions.

Materials and Methods

Following the methods described by Birge and Imhoff, a formalin-fixed human tooth was suspended by a silk thread in the upper one-third of a thousand cc cylinder, containing a 10% solution of disodium-dihydrogen versenate for two months. The solution was not changed, and the cylinder was not disturbed. Alcohol dehydration and paraffin infiltration was employed. A variation of the above procedure was then followed. Using a freshly extracted healthy human tooth, several holes were bored from the surface to the pulp cavity. The tooth was fixed in 10% formalin, and the crown was triple-coated with thin celloidin. It was suspended by the thread, enamel surface downward in the 10% versenate solution. A 1000 cc volumetric flask was used, and was placed in a 56° C paraffin oven and allowed to remain undisturbed for two months. A formalin-fixed mandible of a hamster with soft tissue removed, and containing all the teeth was also triple-coated with celloidin and suspended in versenate solution and allowed to remain undisturbed for the same length of time, but at room temperature for a like period of time. Paraffin sections six microns thick were prepared and stained by hematoxylin-eosin method of both of these specimens.

Results

Sections of the uncoated tooth, decalcified at room temperature show the enamel to be completely dissolved, but the dentine, cementum, and pulp have remained in good condition and stained well. (Figure 1.)

The triple-coated tooth also has the pulp, cementum, and dentine, but in addition, a portion of enamel has remained intact, and displays all the structural detail as described by Orban,[2] in ground sections. (Figures 2 and 3.)

The triple-coated mandible shows no enamel on the teeth, but the bone structures, immature enamel, dentine and pulp of the unerupted tooth are outstanding in their cellular detail and staining qualities. (Figures 4 and 5.)

Discussion

The tooth that was chelated at room temperature did not retain any of the enamel on the completed section, although it may have been present before dehydration was begun. The dentine, cementum, and pulp, however, are in good condition, showing that a prolonged submersion in the chelating solution does not distort the structure.

Figure 1
Low power magnification of chelated tooth without coating of celloidin showing that the enamel has been dissolved.

Figure 2
Low power magnification showing portion of enamel remaining on surface of chelated human tooth.

The triple-coating on the crown surface acts as a brace, to keep the enamel from disintegrating when the tooth is trans-

Figure 3
High power magnification of chelated enamel from tooth that has been triple coated with celloidin.

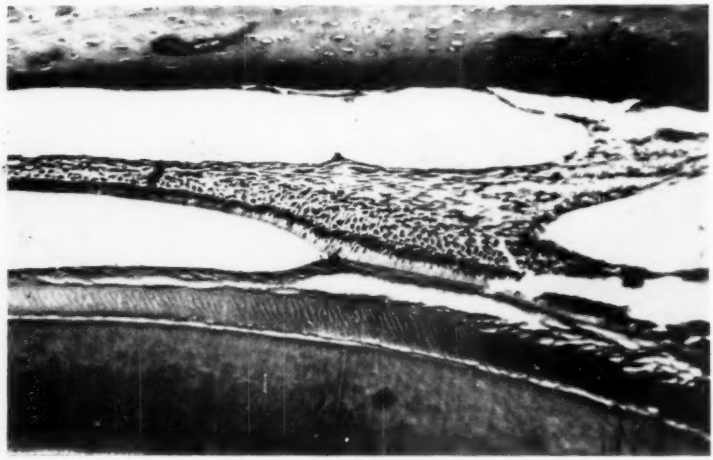

Figure 4
Low power magnification of hamster mandible showing bone, tissue and dentine, plus a layer of immature enamel. Three types of tissue may be sectioned with equal success using the chelating process.

ferred from one solution to another in the dehydration-embedding process. It was also felt this method preserved more of the finer details of the enamel structure, such as rods and rod sheaths,

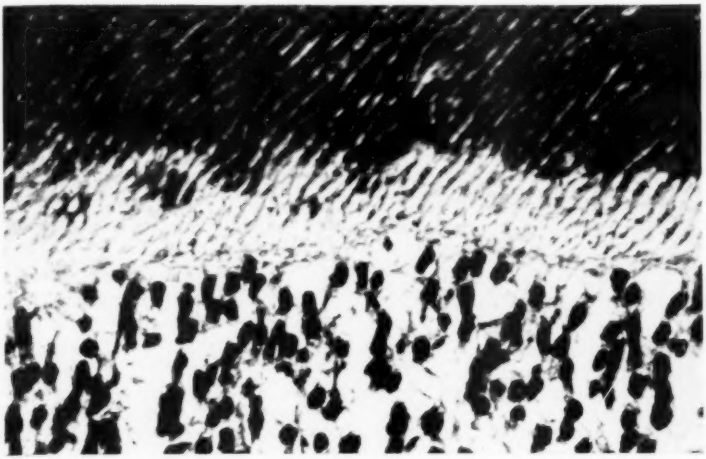

Figure 5
High power magnification showing pulp, secondary dentine, and dentine. The pulp has remained in excellent condition although subjected to long periods in the chelating solution.

than is usually seen in decalcified immature enamel. The mandible shows that cancellous bone, haversian bone, and dentine may be decalcified with the same degree of excellence when they are present in the same specimen. The enamel layer of a hamster tooth is so minute that it would be even more difficult to retain than in the human structure.

Although this procedure did not retain all of the enamel, it did show some progress in this direction. Perhaps some further variation would produce the right combination of factors for a more nearly perfect result routinely.

ACKNOWLEDGMENT

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FUNGICIDAL AND FUNGISTATIC TEST METHODS FOR THE MEDICAL TECHNOLOGIST*

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In testing fungicides, practical methods were explored for the preparation of media containing fungicides and the stability of these preparations in storage. The outline presented below is based on and synthesized from methods used by various investigators, particularly those reviewed by Oster and Golden.[1]

They have stated that the problem in antifungal therapy is to find an effective toxicant that will come in contact with and penetrate the outer layer of the fungus in all its growth phases without being harmful to the host and in a reasonably short enough time to avoid prolonged treatment. They consider the main avenue of antifungal activity to be (1) oxidation, (2) reduction, (3) protein precipitation, (4) enzyme poisoning, (5) deprivation of trace metal action by chelating agents, and (6) competitive inhibition.[2]

Test methods should not be lengthy or require great technical skill or complicated apparatus, so that routine testing and screening of compounds may be done. Fungistatic methods test the growth preventing effect of a substance on a fungus culture as long as the substance remains in contact with the fungus. Fungicidal methods test the killing effect of a substance on a fungus culture after a limited period of contact, following which the substance is completely removed from the cultures which must not have been altered in basic structure by substance or method.

Fungistatic Methods: There are two general methods for estimating the fungistatic effectiveness of chemicals, the Serial Dilution method, and the Agar Cup Plate method. More recently the Disc method has been receiving some attention.

In the serial dilution method, the accurately diluted test substance is incorporated in plates containing Sabouraud's agar. For this test Oster and Golden[3] have modified the method of Schamberg and Kolmer[4] so as to be applicable to alcohol-soluble compounds as well as water-soluble compounds. They did this by limiting the quantity of alcohol incorporated in the agar to 0.1 cc, an amount which exerted no noticeable fungistasis. Plates are poured, each containing 20 cc of Sabouraud's agar into which 0.1 cc of an alcoholic solution of the test substance has been incorporated, thereby diluting by two hundred times the original test substance in alcohol. The surfaces of the plate are streaked with a ten-day-old culture of the test organism, and the plates are incubated for two weeks after which the presence or absence of growth is noted. The highest dilution of the test material

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which completely inhibits growth is regarded as the critical fungistatic dilution.

Oster and Golden[1] further modified this method by incorporating serum in the agar. This was based on the fact that some of the most effective antifungal substances are inhibited by the presence of proteins. Therefore, the Sabouraud's agar medium used in this method should contain at least 10% serum so as to ascertain a possible protein effect. The 10% value is chosen arbitrarily and does not signify that 10% serum is equivalent to the protein content of the human skin.

This fungistatic test method is very valuable in testing of volatile compounds. Its shortcomings are that the test reveals only one value at which the substance is either fungistatic or not, and that nothing is learned of its essential activity or progressing and leveling off to a constant ceiling of fungistasis.[1]

The Agar Cup Plate method, originally introduced by Reddish in 1929, was adapted for fungistatic testing by Burlingame and Reddish in 1939.[5] Serum agar is prepared by adding 2 ml of sterile horse serum to 18 ml of Sabouraud's agar which has been melted and cooled to 40°C. The agar is poured into a 9 cm petri dish, and, when hardened, the entire surface of each plate is streaked with a ten-day-old culture of the test organism. A 2 cm cup is cut from the center of the plate with a sterile cork borer, and approximately 0.8 ml of the test substance dissolved in 95% alcohol or other suitable solvent is pipetted into the cup. The fungistatic activity of a given compound is indicated by a cleared zone around the cup after a growth period of five days at room temperature. The clearance is expressed as a radius segment in mm from the edge of the cup to the beginning of the growth by an average of at least 5 determinations. By measuring several clearances at different dosages, a slope of activity may be obtained using the dosage response curve.

From the dosage response curve an activity coefficient of a fungistatic substance may be calculated. As a standard of comparison a 5 mm zone at 1% concentration of the compound tested is arbitrarily chosen as the activity 1. Using *Trichophyton mentagrophytes* (640 Emmons) as the test organism, the concentration at which a compound exhibits 5 mm clearance divided into the figure 1 gives the fungistatic activity coefficient of this particular substance.[3]

Solvents: Water or saline is a solvent of choice. However, some materials are insoluble in water, so the problem of solvents has to be considered. These should be sufficiently dilute in the final concentrations used, that they will not in themselves have an inhibitory effect on the fungi. Higher inhibitory values are obtained when liquids which increase the solubility are used.[6]

Hillegas and Camp[7] have made fungicidal evaluation of water

insoluble compounds using 25% alcohol, 50% acetone and 80% propylene glycol as solvents. Foley[6] et al used equal parts propylene glycol and water, 2% aqueous triethenolamine and 95% alcohol as solvents.

Fungicidal Test Methods: Altho Kligman and Rosensweig[8] feel that to be useful clinically fungicides need only satisfy the criterion of fungistasis, Oster and Golden[2] believe that at the present stage of our knowledge that the superficial mycoses require true fungicidal agents to be clinically effective. Since fungistatic agents seem to give rise to frequent reoccurrences many tests designed to evaluate the fungicidal power of any substance have been devised.

Two fungicidal methods will be outlined here. The first has been outlined by Emmons[9] in some detail. The test organism should be a strain of *T. mentagrophytes* (*T. interdigitale, T. gypseum*) isolated from dermaphytosis of the foot. A suitable strain, No. 9533, is procurable from the American Type Culture Collection, Georgetown University, 3900 Reservoir Road, Washington, D. C. The strain should sporulate freely on artificial media, the presence of abundant conidia being manifested by a powdery appearance on the surface of a 10 day culture. Conidia of the required resistance should survive a ten minute exposure at 20°C to a phenol dilution of 1:60 but not to one of 1:45.

Spore suspensions should be prepared from the 10 day culture in a concentration of 5 million conidia per ml. The number of conidia is not intended as a safety factor, but was adopted to minimize false negatives resulting from the possibility of picking up loops of material without organisms in the course of the test. It is essential that enough conidia be present in the inoculum to permit the making of subtransfers. Otherwise failure to differentiate between fungicidal and fungistatic action may invalidate the conclusion.

The fungus should be carried on Sabouraud's dextrose agar slants. The same medium should be used in preparing cultures for obtaining conidial suspension, and liquid Sabouraud's should be used to test the viability of conidia after exposure to the fungicide. In caring for fungus strains, whether stock strains or those actually obtained from patients, the following rule should be observed: cultures on Sabouraud's dextrose agar slants should be stored at 2°-5°C. Cultures which have been kept at room temperature or higher for more than 10 to 14 days should not be used as a source of inoculum.

Conidial suspensions for the test should be prepared by planting the inoculum at the center of the agar plate and incubating the cultures at 25° to 30°C for 10 days and not exceeding 14 days. The mycelial mat should then be removed from the agar surface to a flask containing 20-25 ml of a physiological salt

solution per plate by means of a sterile spatula or heavy flattened wire. The conidia are freed from the mycelium by shaking the mixture gently with or without glass beads, and the suspension is filtered so that the hyphal elements are removed while conidia pass through. Sterile absorbent cotton in a sterile funnel is suitable. This should yield 10-25 million conidia per ml. The density of conidial suspension is estimated by counting in a hemocytometer and diluting with a salt solution so that it contains 5 million conidia per ml.

Since it is not absolutely certain which form of growth is responsible for human pathogenicity, Oster and Golden[1] are opposed in principle to those methods which artificially break up the mycelia with glass beads and the subsequent removal of hyphal elements by filtering.

Test: 5 ml portions of each fungicide solution and the phenol control solution are placed in 25 x 150 mm culture tubes and arranged in order of ascending dilutions. These are placed in a 20°C water bath until the temperature of the water bath is reached. With a graduated pipette 0.5 ml of the spore suspension is placed in the first tube of the fungicidal solution, the tube shaken and immediately replaced in the water bath. Thirty seconds later 0.5 ml of the conidial suspension is added to the second tube. This procedure is repeated at 30 second intervals for each of the fungicidal dilutions. After 5, 10, and 15 minute periods of exposure to the fungicide, a sample is removed from each conidia-fungicide mixture with a 4 mm loop and placed in 10 ml of liquid Sabouraud's broth. To eliminate the risk of faulty results due to the possibility of fungistatic action, subcultures should be made. This may be done by withdrawing a loopful of conidia-fungicide mixture, touching it to the surface in a tube of dextrose broth as described above, and immediately immersing it in a second tube of broth. The inoculated tubes are incubated at 25° to 30°C. The final results are read after 10 days altho an indicative reading can be made in 4 days.

A second method adapted by Burlingame and Reddish[5] appears to be superior in that it overcomes the objection of testing fungi like bacteria since the growth of fungus cultures occurs by hyphae matted together into a mycelium, plus a variety of spores, conidia and chlamydospores.

Petri dishes of Sabouraud's agar are inoculated with a culture of the test organism and incubated at room temperature for 5 days, at which time the cultures are cut into 1 cm squares or disks by means of a cork borer 1 cm in diameter. The fungicide to be tested is poured over the surface of the cultures so as to entirely flood the plates. This requires 15 ml or more for each plate. After 5, 15, and 30 minutes one of the squares or disks of culture and agar is removed from each plate and placed in

10 ml of sterile broth. The excess fungicide is washed out of the matted culture by shaking the broth tube lightly for 5 minutes. At the end of this time, the block of culture is removed from the broth and spread, culture side down, over the surface of a sterile slant of Sabouraud's agar. These slants are then incubated at room temperatures for 3 weeks and observed for growth. An effective fungicide should kill the test organisms within 5 minutes.

The test is simple. It uses as test organisms, among others, *T. mentagrophytes*. The solid medium is more adapted to optimum growth conditions of the fungi than liquid media. The washing step avoids a continued fungistatic effect which might still be present when loops of spore culture are transferred in other fungicidal tests.

This test was modified for water insoluble fungicides by Golden and Oster.[1] At the same time, the criteria for fungicidal activity was made more severe. The age of the fungus test culture was raised to 15 days, serum was added to the culture medium, and a 1 minute contact time was followed by an acetone-water mixture washing step. This modified test is specified for alcohol soluble fungicides.

Test: Petri dishes of Sabouraud's agar containing 10% horse serum (blood bank plasma can be used) are streaked with a culture of *T. mentagrophytes* (640 Emmons) and permitted to grow at room temperature for 15 days. On the day of the test the cultures are cut into disks of 1 cm diameter with a sterile cork borer and transferred, with aseptic precautions, to seeding tubes containing 10 ml of the various concentrations of the test fungicides in 95% ethyl alcohol. After a 1 minute contact with the fungicide, the disk is transferred to 10 ml of sterile broth and shaken lightly for 3 minutes to free the matted culture of any water soluble or miscible material. The culture block is then removed from the broth and washed in 10 ml of 30% acetone-water mixture for 5 minutes, thereby removing any fungicide adhering to the mycelia. The acetone-water mixture is not fungicidal in itself even after a 15 minute contact with a 5-day-old fungus culture. Following the acetone washing step, the disk is once more immersed in sterile broth for 2 minutes to remove possible traces of acetone and then spread, culture side down, over the surface of a sterile slant of Sabouraud's agar. These final slants are incubated at 28° ± 1° for 3 weeks and observed for growth.

The test methods so far described deal with the evaluations of pure chemicals. In actual practice, however, the active principal is usually incorporated in some sort of vehicle or carrier. These substances (ointments, waxes, etc.) will exert an influence on the antifungal performance of the active principal.

Burlingame and Reddish[2] describe a method for antiseptic

powders as follows: Liquefy Sabouraud's agar and cool to 45°C. Transfer 20 ml portions into a 9 cm petri dish and allow to solidify. By means of a sterile swab, spread evenly over the surface of the agar a culture of *T. mentagrophytes* (or fungi actually involved in infections) grown on slants of Sabouraud's agar for 5 days at room temperature. To obtain proper distribution the swab should be a loose one, the cotton extending an inch beyond the end of the applicator stick. With a sterile spatula, apply approximately 1 gram of the test powder to the surface of the agar in the center of the plate and cover with a sterile unglazed clay top. Incubate the plates, top up, at room temperature for 1 week.

To determine the effect of organic matter on the fungistatic activity, add 20 ml of sterile horse serum to 100 ml of the Sabouraud's agar and repeat the test.

Disc Method: In keeping with the general trend towards simplification of sensitivity studies, the paper disc method for fungicidal agents is discussed here. Altho there are certain variables that enter into the zone of inhibition test methods, many workers believe the test to be useful for pilot studies.

Kligman and Rosensweig* have outlined a simple quantitative method (disc) for the laboratory assay of fungicides. Altho this method demonstrates fungistatic activity rather than fungicidal properties, these writers believe that to be useful clinically drugs need only satisfy the criterion of fungistasis. They believe this view to be justified since the fungicidal properties of many of the aliphatic saturated fatty acids used in the therapy of dermatophytosis are far less than their fungistatic properties, and at higher pH these substances do not have fungicidal powers at all.

Method: Add 20 ml of Sabouraud's medium adjusted to pH 6 into a sterile petri dish and allow to harden. A suspension of selected organisms is made up to match the density of the Kingsburg-Clark Standard No. 40 (40 mgs %)*. It is not necessary to take undue pains in accurately preparing the suspension as suspensions approximately equal to the standard will give reproducible results. Carpenter[10] did not find that the size of inoculum influenced results except that the plates with lighter inoculum were easier to read.

For making up suspensions of *Torula* and *C. albicans,* some of the growth is removed with a needle and put into water. For *Trichophyton* species, water is added to the tube and the surface scraped with a strong inoculating loop. For *H. Pedrosoi* and *Sporotrichum Schenckii* it is better to grow the organisms in a small flask on liquid medium (shallow layer). Simple agitation of the flask removes large numbers of spores into the medium

* Obtained from Difco.

and samples are pipetted out. Suspensions of mycelial organisms are pipetted before use through a thin layer of gauze to remove clumps; they are then adjusted to the density of the standard.

The age of the culture within reasonable limits was found not to be a factor of great importance. Kligman[8] used 2-3 weeks old cultures of mycelial fungi and one week old cultures of the yeast-like organisms. Carpenter[10] used cultures from 24 hour Emmon's agar slants when testing Candida strains by the disc method.

The petri dish when ready contains a thin superficial layer in which fungus spores are embedded and a 20 ml base layer below. Plates may be seeded using a sterile cotton swab dipped from the suspension of organisms.

To test any particular fungicide sterile filter paper disc 13mm* in diameter are picked up with sterile forceps and dipped into various concentrations of the fungistatic materials. The excess liquid is drained off by touching the disc to the wall of the container and the disc is immediately dropped on the agar surface. Four discs containing four different concentrations of compound are placed on each plate, and after a constant incubation (25°, 29°C or 37°C) the zones of inhibition are measured and recorded. *Torula* and *Candida* plates can be read in 2 to 3 days, while mycelial fungi require 5 to 7 days. The inhibition zones are always more distinct with yeast-like organisms.

This technique can be applied to ointments and insoluble materials can be used. For both water soluble and insoluble materials acetone is used as a solvent as it keeps down bacterial contamination. Kligman and Rosensweig[8] found inhibition zones to be approximately the same with either acetone or water. However, the studies of Hillegas and Camp[7] suggest that acetone penetrates media more readily than water. For ointments Kligman used a standard vehicle of 45% propylene glycol and 55% Carbowax 4000**. In the case of ointments, the paper discs are dipped in melted ointments and placed on the plate in the usual manner. Dilutions of 1:1000, 1:5000, etc., can be prepared from ointments carried in this manner.

It is recognized that the zone of inhibition test does not differentiate between fungicidal and fungistatic action. In the clear zone areas there may be many viable organisms attenuated to such a degree that they were unable to grow. On the other extreme, the organisms may have all been killed by the action of the material being tested. An intermediate condition may exist wherein the organisms have been affected by the chemical so as to lengthen the lag phase, or the organisms can be injured in such a way that, although living, they are slowly dying and will eventually cease to live.

* Disc can be obtained in diameter desired; 6 mm, 13 mm, etc. from Carl Schleicher & Schuell Co., Keene, New Hampshire.
** Carbide and Carbon Corporation.

To determine which condition exists, one can apply the simple technic suggested by Cade[11] of cutting off chunks from the agar at various points in the clear area, and plating them in suitable agar, after first getting the chunks broken up and dissolved in molten agar (43°C) before pouring it into a petri dish. If the organisms are alive, they will grow and form colonies unless prevented from doing so by fungistasis.

Cade[11] has discussed certain variables that enter into the zone of inhibition tests (discs, cups, etc.). Those variables are listed as follows:

(a) The type of procedure—cup or disc
(b) The diameter of the cup or of disc used
(c) The composition and depth of agar and its effect upon the diffusability of both chemicals and organisms through the agar.
(d) The absorptive or retentive properties of the disc
(e) The amount of material on the disc or cups
(f) The manner of incubation (time and temperature)
(g) The pH of agar and test solution

Based on his data on these variables, Cade maintains that zone size is not a direct criterion of the efficiency of the substance being tested, that the relative value of two or more products is not in proportion to zone size, and increasing the amount of active ingredient in a product to make a larger zone does not necessarily make the product more effective. He concludes that a small but definite zone of 2-3 mm might be preferable as indicating a superior product, especially if this smaller zone is retained over a period of time as shown by daily transfers to a new seeded plate. Under these conditions the material possesses and exerts the desired true antiseptic action, and does not decrease in efficiency rapidly, thereby preventing easy reinfection.

Drugs designed for systemic mycoses, as blastomycosis, histoplasmosis, etc., are best evaluated by *in vivo* testing, i.e. animal experimentation. For those desiring to evaluate antifungal agents using experimentally infected animals,[12,13,14] it is useful to establish 50% end points or the LD_{50} dose. The method outlined here is that of Reed and Muench[15] for protective serum. However, if the method is reversed it can be applied to an infective agent.

Serial dilutions are made 1:1, 1:2, 1:4, etc. up to 1:256. Six mice, or whatever number desired, are tested at each dilution and the results recorded in the number dying and surviving. For example, of a total of 12 mice treated with a dilution of 1:16, nine survived at this or higher dilutions, while 3 died at this or lower dilutions, the calculated mortality is 25%. It is assumed that a mouse surviving at a given dilution of serum (or other protective agent) would have survived at a lower dilution. Col-

umn (b) is therefore added from the bottom, and the subtotal for each dilution is entered in column (d) as the accumulated number of mice surviving at this plus higher dilutions. Conversely a mouse dead at a given dilution would have died at any higher dilution. Therefore, column (c) is added from the top and the subtotals in column (e) give the cumulative numbers of mice dying at given dilutions plus those lower. (See following table of Reed and Muench.[15])

TABLE I

(Unabridged) Protective Serum

DILUTION	Alive	Dead	TOTAL		% Mortality
(a)	(b)	(c)	Alive (d)	Dead (e)	(f)
1 : 1	6	0	32	0	0
1 : 2	6	0	26	0	0
1 : 4	5	1	20	1	5
1 : 8	5	0	15	1	6
1 : 16	4	2	9	3	25
1 : 32	2	4	5	7	58
1 : 64	2	4	3	11	79
1 : 128	0	6	1	17	94
1 : 256	1	5	1	22	96

Add (b) up to obtain (d). Add (c) down to obtain (e).

Since highly susceptible animals are occasionally encountered in most types of tests, it is to be expected that these "accidental" survivals and deaths will occur at times at practically any dilution. They should tend to cancel each other if an equal number of dilutions is taken on each side of the end point. This may be accomplished by abridging the original results as has been done in Table II. (Reed and Muench[15]).

TABLE II

(Abridged) Protective Serum

DILUTION	Alive	Dead	TOTAL		Mortality
(a)	(b)	(c)	Alive (d)	Dead (e)	(f)
1 : 8	6	0	14	0	0
1 : 16	4	2	8	2	20
1 : 32	2	4	4	6	60
1 : 64	2	4	2	10	80
1 : 128	0	6	0	16	100

Add (b) up to obtain (d). Add (c) down to obtain (e).

Calculation: Reed and Muench Formula. The 50% point evidently lies between 1:16 and 1:32, but nearer 1:32. It is assumed that since the mortality at 1:32 is 40% above that of 1:16, while the 50% end point is 30% above, the end point is 30%/40% or ¾ of the distance from 1:16 to 1:32. The formula for the proportionate distance of the end point above the dilution giving

next below 50% mortality is:

$$\frac{50\% - (\text{Mortality \% at dilution next below})}{(\text{\% Mortality next above}) - (\text{\% Mortality next below})}$$ will equal the proportional distance (.75)

Example:

$$\frac{50\% - 20\% = 30}{60\% - 20\% = 40} = .75 \text{ distance.}$$

Since dilutions are increasing on a logarithmic scale, it is necessary to obtain the final reading as follows:

Log. of 16 (lower dilution).....1.2041
0.75 proportionate distance x log 2 (dilution factor).....0.2258

1.4299

Sum (log of end point) 1.4299 which makes the end point at a dilution of 1:26.9 or 1:27.

Conversely, if the titer to be found is that of an infective agent "deaths" and "survivals" are merely reversed in direction, the method otherwise remaining the same.

For convenience, a reversed abridged table and formula are prepared below by the writer:

Abridged Table II for Infective Agent

DILUTION	Alive	Dead	TOTAL		% Mortality
(a)	(b)	(c)	Alive (d)	Dead (e)	
1 : 8	0	6	0	14	100
1 : 16	2	4	2	8	80
1 : 32	4	2	6	4	40
1 : 64	4	2	10	2	17
1 : 128	6	0	16	0	0

Add (b) down to obtain (d). Add (c) up to obtain (e).

Similarly the formula should be reversed:

$$\frac{(\text{\% Mortality at dilution next below}) - 50\%}{(\text{\% Mortality next below}) - (\text{Mortality next above})}$$ equals the proportionate distance.

Example: $\frac{80\% - 50\% = 30}{80\% - 40\% = 40} = .75$ proportionate distance.

From this point on the calculations are the same.

Experimental: In view of the fact that three strains of *Aspergillus sp.* had been repeatedly isolated from the sputum of three patients with lung disease, it was requested that sensitivity studies be conducted on these organisms. Since experimental reports on Squibb's Ethyl vanillate (ethyl—4 hydroxy—3 methoxy—benzoate) indicated that some strains of *Aspergilli* were inhibited at therapeutic levels, it was decided to conduct some studies using this vanillic acid ester and, at the same time, apply the various methods previously described. The agar plate

dilution method will be described in detail since it is an easy method for routine testing and screening of compounds.

Test: 1 gram (1000 mgs) of ethyl vanillate was dissolved in 31.25 ml of solution giving a concentration of 32 mgs/ml. Since ethyl vanillate is rather insoluble in water, the weighed quantity was put into a sterile Erlenmeyer flask and heated to 43°C at which point it becomes liquid. By adding 15 ml of propylene glycol at this temperature (43°C) and making up to final volume (31.25 ml) with Sabouraud's broth, the acid remained in solution and did not become cloudy at room temperature. At first the solution was filtered through a Zeitz filter. However, it was found that by using sterile glassware and sterile broth, the solution did not require sterilization.

Dilutions of ethyl vanillate ranging from 2 mgs/ml to .016 mgs/ml were prepared in the following manner: (dilutions and amounts are those selected by the writer and may be varied if so desired).

1 gram (1000 mgs) ethyl vanillate + 31.25 ml diluent = 32 mgs/ml
2 ml of 32 mgs/ml + 2 ml broth = 16 mgs/ml
2 ml of 16 mgs/ml + 2 ml broth = 8 mgs/ml
2 ml of 8 mgs/ml + 2 ml broth = 4 mgs/ml
2 ml of 4 mgs/ml + 2 ml broth = 2 mgs/ml
2 ml of 2 mgs/ml + 2 ml broth = 1 mg/ml
2 ml of 1 mg/ml + 2 ml broth = 0.5 mg/ml.

1 cc of each of the above dilutions is added to 15 ml of melted Sabouraud's dextrose agar and poured into plates. These plates represent the following concentrations:

1 cc of 32 mgs/ml + 15 ml melted agar = 2 mgs/ml
1 cc of 16 mgs/ml + 15 ml melted agar = 1 mg/ml
1 cc of 8 mgs/ml + 15 ml melted agar = 0.5 mg/ml
1 cc of 4 mgs/ml + 15 ml melted agar = 0.25mg/ml
1 cc of 2 mgs/ml + 15 ml melted agar = 0.125 mg/ml
1 cc of 1 mg/ml + 15 ml melted agar = 0.06 mg/ml
1 cc of 0.5 mg/ml + 15 ml melted agar = 0.03 mg/ml.

Plates were poured, allowed to harden, seeded with suspensions of the organisms and incubated at 26°C. Results were recorded at 2, 5 and 10 days. Suspensions of organisms were made in various ways and using cultures varying in age from 3 to 10 days. Various methods of seeding were employed. Two sets of controls were run, (1) using agar alone, and (2) by incorporating propylene glycol into agar in the same concentrations as in the test. There was no inhibition or slowing down of growth in the latter as compared with the control tube. Sensitivity results were the same for each organism in spite of the variables mentioned. Readings taken after 2 days incubation uniformly read 1 dilu-

tion lower concentration than those taken after 5 days incubation.

Later the method was modified so that 100 ml volumes of agar and fungicide were prepared for each dilution, and approximately 10 ml quantities added to sterile screw cap tubes, slanted, and stored in the refrigerator. Test showed that these tubes gave the same results when used after being stored in the refrigerator for 1 month as when used immediately after preparation. Tubes prepared from the original dilution (32 mgs/ml) of ethyl vanillate which had been (1) frozen, (2) left at room temperature, and (3) left in the refrigerator (2°-5°C) for a period of two weeks, gave the same sensitivity readings as when freshly prepared.

Similar studies with various organisms were conducted testing Stilbamidine*, Propamidine*, and Mycostatin** (Lot No. St695-714 115 B). The first two substances appeared stable after 1 month of storage, while Mycostatin was less fungistatic after 8 days. Stilbamidine gave a clear solution when dissolved in mycophil broth, but was cloudy when dissolved in nutrient broth, probably due to the difference in pH of the broths.

Results of fungicidal tests, Agar Cup Plate tests and other studies being conducted are not complete, so they will not be reported at this time. Studies employing the disc method have been disappointing thus far as results have not always been reproducible and were seldom in a therapeutic range.

Summary: In view of the fact that increasing numbers of medical technologists are becoming proficient in identifying fungi, and the apparent increase in fungus infections following prolonged antibiotic therapy, the problem of fungicides is becoming an urgent one. Many of the larger antibiotic houses are constantly searching for effective fungicides and one has only to read the literature to realize how much work is being done in this field.

Although at present the problem is viewed with pessimism by many investigators, it will be unusual in this investigative age if the problem is not solved.

In order that the medical technologist may be prepared to meet the challenge, basically simple fungicidal and fungistatic methods have been outlined for quick reference and for the routine testing and screening of fungicides.

A method for the determination of the LD_{50} dose or 50% endpoint is described.

Fungistatic studies on Ethyl vanillate, Stilbamidine, Propamidine, and Mycostatin (Nystatin) are briefly discussed.

* Supplied by the Wm. S. Merrell Co., Cincinnati, Ohio.
** "Mycostatin" trade name of E. R. Squibbs & Sons, Div. of Olin Mathieson Corp.

"Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of her own study and do not necessarily reflect the opinion or policy of the Veterans Administration."

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TECHNIQUE FOR THE DETERMINATION AND EVALUATION OF THE SERUM PROTEINS BY TURBIDIMETRY*

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In May 1955 a paper[1] written by Ivan Parfentjev and Mary Louise Johnson outlined the complete protein pattern and its changes in malignancies as compared to health. These changes were then described for use in the diagnostic clinic.

In short, they found indications of a consistency of normal protein patterns in healthy individuals throughout life. There were instances of abnormal protein patterns found predominantly in diseases. As for the validity of their findings, only in the late age group of from 80-90 years were there found abnormal protein patterns in apparently healthy individuals. This figure is as low as 17%. The importance of the determination of protein patterns is supported by finding abnormal patterns in apparently healthy individuals who were subsequently to develop the clinical indications of a malignancy. Such was seldom found in ambulatory patients with various non-malignant conditions.

By contrast, patients with advanced chronic diseases had protein patterns characterized by a much more profound deviation from normality due to such factors as cachexia, (a depraved condition of malnutrition), chronicity and therapy.

Therefore it can be easily seen that an altered protein pattern can be of a help in screening for malignancies when none is suspected clinically.

Research in the subject of the afibrinogenemia of pregnancy and the development of a commercially available injectable form of fibrinogen for its treatment has made it necessary to develop a rapid micro method of assay. Turbidimetry suits this purpose because of its speed, the small amount of plasma needed and the reliability of the results.

Fractions considered:

To determine the individual protein pattern there are six considerations. 1.) total protein, 2.) total globulin, 3.) albumin, (which is arrived at by the difference between the total protein and the total globulin), 4.) euglobulin, including the beta globulin. 5.) pseudoglobulin (total globulin minus euglobulin, and which is largely alpha globulin, some beta globulin and mucoglobulin) and 6.) the acid precipitable protein at pH 5.1.

The assay of fibrinogen is not necessary to evaluate the pro-

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

tein pattern but its significance in other diseases and conditions is well recognized.

Those changes which are indicative of a malignancy:

1.) An increase in the total globulin, 2.) a decrease in the albumin, (both as related to the amount of total protein), 3.) a reverse in the albumin globulin ratio, 4.) an increased pseudoglobulin and 5.) an increased acid fraction precipitated at pH 5.1.

In afibrinogenemia the lower concentrations of fibrinogen to 50-150 milligrams percent indicate that fibrinogen replacement therapy is needed.

Because of individual differences in total protein it is not possible to establish one set of values to be considered normal, but because of a normal range variance, levels of concentration within each range of total protein had to be established as it was by Parfentjev and Johnson.[1] ". . . in proteinemic samples all fractions tend to be low, thus a protein fraction might fall within a normal range but be abnormally elevated when compared to other fractions of the same proteinemic sample. To guard against such misleading values, average normal patterns and limits were established at each of the six different levels of total protein. All normal samples which fall within the range of a certain total protein were distributed so that the frequency with which concentrations of specific fractions were above or below an arbitrary value might be determined. The frequency established the relative significance of maintaining a specific subfraction above or below a given value. Thus at a given certain concentration of total protein, one fraction will weigh the pattern more than another."

The subfractions were given numerical values for the evaluation. If the fraction falls within a normal range of concentration according to the group in which they are placed by the concentration of the total protein, credits are given. Any sample which has two-thirds of the total number of normal credits is rated as a normal serum. There is a corresponding table to evaluate the abnormal values demonstrated by the fractions. A key was set up for the evaluation of normality. It was originally published by Parfentjev and Johnson.[1]

The method of determination utilizes the photoelectric measurement of a precipitated protein in ammonium sulfate suspensions by turbidity. It is standardized by using specimens of serum, the content of which is determined by the Kjeldahl method of analysis. From these a constant factor is determined utilizing a spectrophotometer. By reading the optical density on the upper scale of the coleman spectrophotometer the necessity of creating a curve is eliminated. The optical density and the concentration have a straight line relationship. Therefore a constant factor K is possible.

"Apparently the kind of precipitate and the degree of turbidity depend on the salt concentration rather than the size and the molecular weight of the protein molecule."[3]

Materials used for the standardization of the individual spectrophotometer should be obtained in as pure form as possible. If the prepared standards of Parfentjev are not available, the following are acceptable: pooled human plasma, of which the protein content has been determined by the Kjeldahl method, serum globulin, gamma globulin, immune globulin, diphtheria and tetenus antitoxins, serum albumin, fibrinogen and other pure protein fractions which are commercially available. For the standardization of the acid insoluble proteins which were found to have a significance in the total protein pattern, large quantities of serum were used to precipitate the fraction which was collected by centrifugation, redissolved at neutrality and their protein concentration determined by precipitation with ammonium sulfate and the subsequent measurement of the total protein content existing. The average concentration was found to be 0.5 grams/100 mls. of serum, but it ranged from 0.3 to 0.7 grams percent normally. The pH chosen for this precipitation was 5.1.

The dispersed precipitates are very stable in preparation. For this reason it is possible to prepare pure standards and store them. They can be used to determine the subjective error as well as in finding the constant k for each fraction. Refrigeration of the standards is not necessary, but they must be sealed against contamination and the adsorption or loss of water.

In finding the constant factor for all of the fractions the optical density x the constant = concentration, or stated like this:

$$\frac{\text{concentration}}{\text{o.d.}} = k \text{ (Each fraction has a different factor.)}$$

When doing large volumes of testing using ammonium sulfate prepared in liter quantities as recommended, it must be kept in sealed containers because the absorption of water will change the factor. This consideration is most important when using glassware such as beakers and pipettes. Of course the reagent never can be returned to the stock bottle.

Ammonium sulfate may be buretted into the cuvettes but it must have been newly placed in the burette, or the method of filling the burette must protect the solution from the air. Exposure to air for more than one hour introduces a considerable error. No precautions out of the ordinary are called for by the acetate buffer. The following table describes the content of the reagent for each specific fraction and the components of that fraction.

This measurement of turbidity caused by the precipitation of protein is accurate to within $\pm 5\%$ using either serum or plasma.

Proteins	Gms. Merth.	% Sol. of $(NH_4)_2SO_4$	$(NH_4)_2SO_4$ in Grams	Grams NaCl	Grams of Gum Ghatti	H_2O	Fraction Obtained
Fibrinogen	.025	12%	133.33	10.0	5.5	qs. 1L	Fibrinogen
Euglobulin	.025	21%	216.07	10.0	5.5	qs. 1L	Alpha, beta and gammaglob. and fibrinogen
Total globulin	.025	27%	270.80	10.0	5.5	qs. 1L.	Alpha, beta, gamma and mucoglobulin and fibrinogen
Total protein	.025	50%	500.00	10.0	7.0	qs. 1L.	Fibrinogen, albumin and the globulins
Acid fraction	Use an acetate buffer pH 5.1						The acid insoluble proteins

Ten cubic centimeters of blood are collected by venipuncture. The serum is acceptable if only the protein patterns are to be done. If plasma is to be collected in order to determine the fibrinogen content, 0.5 ml. of a 4% solution of sodium citrate ($2H_2O$) is a good anticoagulant to use. It must be in a dry form.

The whole blood is centrifuged for twenty minutes at 2500 rpm. The serum or plasma is decanted and the supernatant is recentrifuged for a short time if necessary and again decanted. Over exposure to the heat of the centrifuge is injurious to both the proteins and to the fibrinogen in particular.

After decanting it is possible to store the blood for more than eight hours if the method of collection and handling has been sterile The material is refrigerated to keep it over night or longer. The tubes must be stoppered to prevent evaporation and contamination. Keep in mind that it is a good bacterial medium.

If there is evidence of chyle or a hemolysis it is necessary to prepare a readout. Actually the blanks for color should be prepared routinely.

The Coleman Senior Spectrophotometer is preferred for this method but a junior model is acceptable. The junior is usually more stable but less sensitive. An arbitrary wave length of 450 λ was chosen for all the fractions because it demonstrates the best range of sensitivity. Use optically ground cuvettes, 19 x 105 mm. Because of the tendency of ammonium sulfate to pick up water and because only a small amount of it will alter the accuracy of the test so markedly, the cuvettes must be absolutely clean and dry. After use they should be rinsed in tap water and then washed. Rinse with distilled water and dry with acetone or alcohol-ether rinses. They are inverted to drain. All the usual precautions taken in the use of the spectrophotometer are taken, to avoid scratches in the glassware, fingerprints, etc.

Dilutions of the serum are made volumetrically. Use normal

saline. The recommended dilutions are a 1:6 dilution for the total protein and a 1:3 dilution for the other fractions. No dilution is necessary for the determination of fibrinogen. A 1:2 dilution can be used for the acid fraction.

The diluted material is drawn up in a Kahn blue line pipette to the 0 mark. The bottom of this pipette is placed on the bottom of the cuvette and the meniscus is lowered to the desired gradation. If too much is pipetted do not try to draw the fluid back into the pipette. Repeat the operation. Ten cc. of the proper concentration of the ammonium sulfate is added, using a volumetric transfer pipette or a burette. Allow the salt to wash down the sides of the cuvette, shaking the mixture constantly while adding it. This is the trick to this determination. If the salt mixture is blown into the serum, large flakes will form and will appear as a flocculation in the fluid rather than an evenly dispersed turbidity. Do not touch the protein material with the delivery tip. By this technique a homogenous mixture is assured and there is less error. If flocculation occurs in the face of the proper technique, it is probable that the concentration of the protein is high and further dilutions should be made. Be sure to include any dilutions when computing the final concentrations. Never read a flocculated sample. The protein cannot be blown into the salt, as with a Sahli pipette, because this flocculation occurs. Prepare each fraction in duplicate. Allow the sample to attain its full turbidity before reading or there will be a difference of from 0.005 to 0.10 on the logarithmic scale. Three minutes is usually sufficient. Always shake the tubes in a rotary motion before reading the optical density.

The duplicate samples are compared to a blank consisting of saline in an aliquot amount equal to that of the diluted sample plus ten milliliters of the salt used to precipitate the protein.

A color correction is prepared at the same time. It consists of an aliquot of the diluted serum plus 10 ml. of .85% NaCl read against a blank of 10 ml. of sodium chloride. The optical density obtained is subtracted directly from the optical density of the sulfate precipitated sample. The color blank usually reads below 0.100 if the plain saline is set at 0.

Formula for the calculation—the average optical density of the two samples—the optical density of the color blank = the corrected optical density. The corrected optical density x the dilution factor x the constant K for that fraction = the percent in grams per 100 ml. blood for that fraction.

In standardizing the spectrophotometer it is best to keep certain elements standard. For example, the wave length is most sensitive at 450 λ. The 0.12 or 0.15 ml. of diluted material used to produce a turbidity in the salt solution should not be varied because it will dilute or underdilute the reagent. There must

be a variable however and the dilution of serum can provide this flexibility. Use the one which will render the most reproducible readings, and which can be delivered in the prescribed aliquots. For total protein a dilution of 1:6 is acceptable. The concentration of the protein is usually then at about 1.1%. For total globulin and euglobulin 1:3 is the usual dilution. The acid fraction calls for 0.10 ml. serum in about 10 ml. acetate buffer, so any dilution which renders the amount easy to handle with a pipette and which delivers 0.05 ml. is usable. 1:2 is best.

Ten ml. of the acetate buffer at pH 5.1 are buretted into the cuvette exactly as are the ammonium sulfate solutions. Use the same technique. No particular precautions are necessary with the buffer other than purity and the accuracy of its pH. The observed optical density is corrected and then multiplied by the factor for dilution. The O.D. is then multiplied by the constant for that fraction.

All the readings on the spectrophotometer have their greatest accuracy between .200 and .400 on the upper scale.

For the exact details on the standardization of the spectrophotometer using the method of Parfentjev and Johnson consult The Yale Journal of Biology and Medicine, Vol. 25, No. 2, November 1952. Here the materials used are described and the values obtained as compared to other acceptable methods are reviewed.

Evaluation—briefly ". . . the consistency, accuracy and simplicity of this rapid micro method for serum fractionation emphasize the possibility of an extensive clinical use as a facile routine procedure. Moreover the method is applicable to the study of aberrant protein patterns."[2] The importance of this method in rapidly determining the fibrinogen content has been mentioned.

The materials used are a Coleman spectrophotometer and cuvettes 19 x 105 mm., distilled water, sterile saline collection bottles, dry anticoagulant, centrifuge tubes, stoppers, Kahn blue line pipettes, 10 ml. volumetric pipettes, the ammonium sulfate solutions and the acetate buffer.

Since a degree of accuracy is impossible without the proper salt concentration the method of preparing the standard ammonium sulfate solution and its preservation is needed. The ammonium sulfate and sodium chloride are mixed with distilled water. Gum ghatti is then added to serve as protecting colloid and then the volume is brought to the one liter mark with the distilled water. The pH is adjusted to 7 using 10 N NaOH as indicated by using 0.02% phenol red. Since there is a danger of microbial growth, a dilution of merthiolate should be included. In the 50% solution the concentration is high enough to prevent

Name	Amt. of Salt	Salt Conc.	Amt. of Serum	Dilution of Serum	Amt. of Dilution	Blank
Total protein	10cc.	50%	0.02cc.	1:6	0.12cc.	10cc. 50% Ammonium sulfate and 0.12cc. saline
Total globulin	10cc.	27%	0.05cc	1:3	0.15cc.	10cc 27% Ammonium sulfate and 0.15cc. saline
Euglobulin	10cc	21%	0.05cc.	1:3	0.15cc.	10cc. 21% Ammonium sulfate and 0.15cc. saline
Fibrinogen	10cc	12%	0.10cc.	none	0.10cc.	10cc. 12% Ammonium sulfate and 0.10cc. saline
Acid fraction	10cc. Acetate buffer	....	0.10cc.	none	0.10cc.	10cc. of the Acetate buffer and 0.1cc. of saline

Criterion and Credits for the Evaluation of Normal Sera

	Total Protein		Total Globulin		A/G Ratio		Pseudo-globulin		Acid Fraction	
Group	Range	Credit	Range	Credit	Range	Credit	Range	Credit	Range	Credit
A.....	<6.0	3	<2.8	3	>1.1 >1.2	2	<0.6	4	<.39	4
B.....	6.0 6.4	5	<2.8	5	>1.1 >1.2	1 2	<0.6	1	<.39	2
C.....	6.4 6.8	5	<3.0 <2.4	2 3	>1.3 >1.5	1 2	<0.6	1	<.39	2
D.....	6.8 7.2	5	<3.0 <2.5	2 3	>1.3 >1.5	1 2	<.06	1	<.39	2
E.....	7.2 7.6	5	<3.0 <2.6	2 4	>1.3 >1.5	1 1	<0.6 <0.5	1 1	<.42	1
F.....	>7.6	3	<3.25 <2.7	2 5	>1.4 >1.6	1 1	<0.8 <0.7	1 1	<.58 <.48	1 1

Criterion and Credits for the Evaluation of Abnormal Sera

	Total Protein		Total Globulin		A/G Ratio		Pseudo-globulin		Acid Fraction	
Group	Range	Credit	Range	Credit	Range	Credit	Range	Credit	Range	Credit
A.....	<6.0	3	>2.9	3	< .97	2	>.6	4	>.39	4
B.....	6.0 6.4	5	>2.9	5	<1.28	1	>.6	2	>.44	3
C.....	6.4 6.8	5	>3.0	5	<1.31	1	>.6	2	>.44	3
D.....	6.8 7.2	5	>3.0	3	<1.35	1	>.6	2	>.42	5
E.....	7.2 7.6	3	>2.9	2	<1.37	1	>.6	5	>.42	5
F.....	>7.6	2	>3.25	1	<1.39	3	>.6	5	>.46	5

any growth. The preparation is allowed to stand for 24 hours and then is filtered to remove the excess gum ghatti.

The acetate buffer is prepared according to standard procedure.

Determine the group in which the sample falls according to its content of total protein. Credit it with the designated value. If the concentrations of the corresponding fractions do not fall in the prescribed range, ignore the value. If two ranges are given and the sample satisfies both of them, credit both. Record the total credits. Do the same for the sample on the chart evaluating abnormal characteristics. Malignancy is expressed by an overwhelming number of credits for abnormality as compared to normality.

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ATTENTION!

Our attention has been called to a misunderstanding of the author in the article entitled *A Comparative Study of Oxyhemoglobin and Cyanmethemoglobin Determinations by Photometric and Spectrophotometric Methods* which appeared in Volume 23, No. 1, January-February 1957, by Norman J. Duchateau. The free samples of the standard mentioned in the footnote on page 22 are not available as stated. Please do not write for these.

SAFETY PRACTICES IN THE CLINICAL LABORATORY

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Most general laboratories are considered to be safe places in which to work. This is because of technical training and indoctrination into safety practices from the beginning of the laboratorian's career.

In the clinical laboratory of the hospital, the exposure to human secretions and excretions, toxic chemicals and gases, contaminated specimens, the danger of contamination by infectious organisms, point to this area as a constant source of hazards to the hospital personnel.

Accidents do occur here but in most instances can be prevented if a definite program of precautions or safety practice is set up and followed intelligently. Fundamentally, very little change occurs in the rules of safety, and when these rules are absorbed, the response of the individual is automatically controlled to contain any type of hazard.

An educational program and rigid rules, along with the recognition by the head of the laboratory that a plan of this type must be in effect at all times are necessary in an attack against laboratory accidents.

The degree of safety practice completed in any laboratory is in direct proportion to the attitude of the person in charge. A demand for complete recognition of these factors by the administration will contribute to the reduction of accidents in the clinical laboratory.

The major sources of accidents in the hospital laboratory can be summarized if one divides the laboratory into departments as is the practice in the larger institutions as follows:

1. Bacteriology and Parasitology
   a. open specimen containers
   b. splattering of material from inoculating needles
   c. aerosols
   d. use of unplugged pipettes
   e. improper sterilization or disposal of specimens
   f. contempt for any organism
   g. lack of availability of transfer hood for virulent cultures
2. Serology
   a. contaminated serum samples
   b. living antigens
   c. glassware
3. Histo-pathology
   a. infected tissues prior to fixation
   b. autopsy and microtome knives
   c. flammable materials i.e. solvents and waxes

4. Chemistry
   a. toxic chemicals and gases
   b. acids and alkalis
   c. electronic instruments
   d. glassware
   e. radioactive chemicals
5. Hematology
   a. improper sterilization of lancets
   b. glass slides and cover slips, pipettes

The prevention of accidents is the one important step in organizing a safety campaign in the hospital laboratory. Starting with storage facilities in their relation to proper spacing of materials and ending with directions for disposal of specimens is a good beginning.

Adequate warehousing and proper ventilation including a door or window to the outside, plus a blower system to change the air in the entire room rapidly are imperative. For storage of volatile solvents, fire doors, safety lamps and fire extinguishers should be provided.

Of great import is the positioning of materials for storage. Heavy containers should be placed as close to the floor as is possible, without projection of any type of equipment beyond the shelf front. Gas cylinders should be stored in such a manner so as to prevent rolling or falling, be capped tightly and removed from flame and heat. The greatest precaution in any storage room is good housekeeping since the accumulation of dirt and waste is a definite fire hazard.

Modern complex instrumentation with high voltages can be a constant source of danger to the technologist. Thus voltage and makeshift assemblies can combine to cause trouble. The do's and don'ts of protection against electrical shock in the home and industry are equally applicable in the clinical laboratory.

The insignificant cuts and slight burns are the most common types of injuries met in the clinical laboratory. Most individuals dismiss these without a thought since they occur frequently. These untreated and exposed injuries can and at times will lead to serious consequences by allowing for the entrance of bacteria and increase the chance for tissue damage by corrosive compounds.

The sputtering and splashing of acids, alkalis and oxidizing agents on skin and clothing should be flushed immediately with copious amounts of water.

The availability of safeguards against burns, both chemical and heat such as beaker covers, safety tongs, chemical handcreams, gloves, goggles and aprons should not be overlooked in providing protection for the technologist. The safety conscious technologist uses these tools to such an extent that they become

second nature. The use of goggles and also limiting of exposure to extra visual light is strongly recommended.

The removal of harmful vapors and gases by means of a fume hood which provides for adequate displacement of air through multiple vents to the outside is mandatory. The inhalation of small amounts of toxic gases can cause or preclude a serious illness. Ventilating systems are considered adequate and efficient only when noxious vapors are removed rapidly to the outside without danger of re-entering the structure.

With the expansion of radio isotope studies, monitoring systems should become a standard installation in the hospital laboratory.

In handling materials and cultures, especially from suspected cases of tuberculosis and certain fungus and virus diseases, a bacteriological hood is strongly recommended. This type of apparatus has arm holes for working under glass, the enclosed area is flooded with ultraviolet light and is equipped with a heated exhaust system having a temperature of sufficient heat to kill any micro-organisms that are drawn through it. Another type of cabinet has a built-in filter to remove organisms from the system prior to reaching the external duct work. Air entering the cabinet is filtered to prevent outside contamination.

In routine work with specimens, the ordinary precautions and instructions given in an introductory course in bacteriology should be followed. With improvement in glassware such as in the cotton plug and taper top pipettes, the plugging of transfer pipettes has been made less time consuming and allows for a safer approach in the handling of liquid specimens or cultures in a liquid medium.

The productivity and proficiency of the histologic technician has been increased by automatic tissue processing equipment and knife sharpeners. The former reduced solvent exposure in the open room by individual covers or sheets. The automatic and semi-automatic knife sharpeners are so designed that handling of the knife itself is at an absolute minimum, thereby reducing chances for cutting.

The disposal of volatile and corrosive substances can be handled by proper training of janitorial and washroom help or by the technologists themselves. Three separate cans or jars should be provided and be labeled for broken glass, acids and inert chemicals. Volatile, poisonous, radioactive gases and liquids require a tightly stoppered, clearly labeled container. Cyanides in common usage should be checked from storage shelf to final disposal. In this manner, non-technical help are protected.

The continuing use of the more powerful acids such as perchloric for digestion purposes brings out the explosive dangers involved with compounds of this nature. Hoods of special design

and made from non-organic and non-porous duct work with arrangements for frequent washing down are necessary. This is provided for by means of a perforated stainless steel tube running the length of the top of the hood and behind the baffles. Duct work should never be connected to a system serving any other type of fume hood or equipment. Individuals working with materials of this type should wear protective clothing as well as an eye shield since one drop may cause the loss of an eye. Discolored perchloric acid should be discarded by diluting it 10 times with water and pouring it into an acid sewer followed by thorough flushing.

The disposal of bacteriological specimens other than cultures in most instances can be accomplished by incineration, or if contained in glass, the material should be autoclaved prior to disposal.

Chemical sterilization of protein containing solutions at times is inadequate due to the dilution of the germicide or to the precipitation of the protein, preventing access of the bactericidal agent to the organisms. Definite concentrations of an agent must be maintained to kill organisms in this manner. The concentration required will vary with the organisms encountered and with existing conditions.

The most efficient way of sterilizing most materials required for use in the clinical laboratory is by steam under pressure. With the advent of fully automatic equipment, the efficiency of this method especially in regard to glassware has improved. The same precautions exercised in bacteriological methods should be followed in serology wherever possible.

The use of individually packaged and sterilized disposable lancets has brought a revolutionary change in the hematology laboratory by replacing the old style spring operated points or knife blades. The latter, between uses were always rinsed with alcohol before being used on a succeeding patient with sterility never being assured. Bevel ground glass slides and dispensers for cover slips have aided in the decline of small cuts from these sources.

Emergency charts (available free of charge), should be in every laboratory. These are condensed for quick reference and understanding. Their use has resulted in saving of eyes and recovery from acute poisoning by prompt and adequate treatment. The cost of safety is small when compared to the price of an accident to personnel with resultant time loss, as well as replacement problems, a well known situation at the present time in the clinical laboratory of all hospitals.

A SENSITIVE REAGENT CAN BE MADE IN ANY LABORATORY FOR THE DETECTION OF OCCULT BLOOD

A. T. BARTHOLOMEW

Houston, Texas

It is the desire of every technologist to have at his finger tips a rapid sensitive reagent to demonstrate the presence of occult blood. A dry mixture of powders to take the place of liquid reagents can be made in your own laboratory.

Simply take a glass cylinder and pour reagent grade benzidine, (for occult blood test), into it until it can be leveled off at 4 ml. mark. Next pour sodium perborate on top of the benzidine until it can be leveled off at the 10 ml. mark. Put your hand over the top of the cylinder and tilt the cylinder one end up and then the other until the powders are well mixed. This is the reagent powder ready for use.

Procedure:

A. Add a drop of glacial acetic acid to a drop of the specimen to be tested.
B. Dispense a small amount of powder, from the point of a knife blade, into the same specimen.
C. Instantly a bright blue color appears if blood is present in concentrations of one part in 40,000 parts. If less than one part in 200,000 parts of blood is present, the reaction will be lighter and will develop in about 30 seconds.

Each lot should be tested for sensitivity. If the best grades of reagents are used, you will find that 8 or more RBC/HPF. will give a definite reaction.

Reactions:

Trace—faint green
1 Plus—green
2 Plus—greenish blue
3 Plus—blue
4 Plus—deep blue
Neg.—brown or gray

Reagent is stable for years.

Applications:

A confirmation for students who confuse crystal forms with RBC.

For examination of gastric content, vomit, and other biofluids.

Physicians office for any detection of Hb. he may wish to question.

Examination of cloth, soil, and instruments for medical jurisprudence.

Limitation:

Should not be recommended for examination of stools unless the patient has been on a strict diet. Its sensitivity is capable of detecting blood devoured with meals. It can be altered for this by mixing tartaric acid powder in it and omitting the acetic acid in the procedure.

AMONG THE NEW BOOKS

CLINICAL LABORATORY DIAGNOSIS. 5th Edition. By Samuel A. Levinson, M.S., M.D., Ph.D., Director of Laboratories, University of Illinois Research and Educational Hospitals, Chicago, Illinois; Professor of Pathology, University of Illinois College of Medicine, and Robert P. MacFate, Ch.E., M.S., Ph.D., Chief, Division of Laboratories, Board of Health, City of Chicago; Director of Laboratories, Municipal Contagious Disease Hospital, Chicago, Illinois, and five collaborators. Lea & Febiger, 1956. 1246 pages. 244 illustrations and 13 plates, 11 in color. 142 Tables. $12.50.

Brought up to date, this standard reference volume has been made more valuable than ever to the practicing medical technologist. Not only are detailed methods given, with comprehensive pertinent discussions of the subjects in question, but sections are devoted to a correlation of findings in health and disease, and to concise interpretation of application of basic science principles to laboratory medicine.

Much of the book has been rewritten; chapters and sections are entirely revised. That on Hematology includes many of the newer concepts so arranged that the relation of techniques to specific blood diseases is logically shown. In some instances related methods are briefly reviewed with more detail for those preferred. Nomenclature recommended by the National Institutes of Health is the terminology of the revised chapter on Blood Banking. Recent knowledge of sugar and protein metabolism, as well as micro techniques, is included in the section on chemistry. There is also new material in those on serology, histology, legal medicine and toxicology, and tropical diseases. The authors have succeeded in providing the laboratory technologist and physician with a most practical text.

CLINICAL LABORATORY METHODS. 5th Edition. By W. E. Bray, B.A., M.D., Consulting Laboratory Director, Martha Jefferson Hospital, Charlottesville, Va.; formerly Professor of Clinical Pathology, University of Virginia and Director of Clinical Laboratories, University of Virginia Hospital. The C. V. Mosby Company, St. Louis, 1957. 731 pages. 124 illustrations, 18 color plates. Tables. $9.75.

Long a favorite in the laboratory, this book is brought up to date to include brief treatises on Salk poliomyelitis vaccine, the use of radio active chemicals in diagnosis of disease, as well as others of the newer techniques and procedures. In each section there is a discussion with references and a comparison of methods, to allow for a choice. Most of the formulas for reagents, including indicators and stains, are to be found in a convenient separate chapter which is fully indexed.

(Continued on Page 177)

THE EVALUATION OF BROMSULPHALEIN FOLLOWING CHOLECYSTOGRAPHIC STUDIES*

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The question has been raised in our laboratory concerning the accuracy of bromsulphalein (Hynson, Westcott and Dunning brand of Sulfobromophthalein sodium, U.S.P.) determination following Telepaque (Trademark, U. S. Pat. Off., Winthrop-Stearnes, Inc.) cholecystographic studies. Lowman and Stanley[1] state that delayed removal of bromsulphalein following administration of telepaque for twelve to eighteen hours may occur in patients with hepatic diseases and occasionally in normal individuals. In this paper the results are presented of the study of nineteen patients referred for cholecystographic studies with possible gall bladder pathology and of nine healthy student nurses.

Three bromsulphalein[2] determinations and parallel cephalin cholesterol flocculation tests[3] were performed on each of the nineteen patients, and two bromsulphalein determinations with parallel cephalin cholesterol flocculation tests on each of the nine nurses. The determinations were made as follows: (a) twelve hours preceding and twenty-four hours following the administra-

TABLE I

Comparative Retention of Bromsulphalein Following Telepaque Administration Changes in Cephalin Cholesterol Flocculation

Nineteen Patients

PATIENT	12 Hours Before TA*		24 Hours After TA		2 Weeks After TA	
	BSP	Ceph. Chol.	BSP	Ceph. Chol.	BSP	Ceph. Chol.
1.	16%	neg.	16%	1+	14%	1+
2.	11%	neg.	8%	neg.	....	....
3.	2%	neg.	3%	neg.	2%	neg.
4.	9%	neg.	22%	neg.	6%	neg.
5.	2%	neg.	4%	neg.	1%	neg.
6.	6%	neg.	8%	2+	2%	neg.
7.	1%	neg.	1%	neg.	1%	neg.
8.	2%	neg.	1%	neg.	1%	neg.
9.	3%	neg.	3%	neg.	2%	neg.
10.	3%	neg.	4%	neg.	1%	neg.
11.	1%	neg.	2%	neg.	1%	neg.
12.	2%	neg.	2%	neg.	....	....
13.	2%	neg.	2%	neg.	1%	neg.
14.	2%	neg.	2%	neg.	1%	neg.
15.	6%	neg.	7%	neg.	2%	neg.
16.	2%	neg.	3%	neg.	2%	neg.
17.	1%	1+	2%	2+	1%	3+
18.	8%	neg.	8%	neg.	2%	neg.
19.	1%	neg.	2%	neg.	....	....

* TA Telepaque Administration.

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

TABLE II
Comparative Retention of Bromsulphalein Following Telepaque Administration Changes in Cephalin Cholesterol Flocculation Nine Student Nurses

PATIENT	12 Hours Before TA*		24 Hours After TA	
	BSP	Ceph. Chol.	BSP	Ceph. Chol.
1.	1%	neg.	2%	neg.
2.	1%	neg.	1%	neg.
3.	1%	neg.	1%	neg.
4.	1%	neg.	3%	neg.
5.	1%	neg.	2%	neg.
6.	1%	neg.	4%	neg.
7.	1%	neg.	5%	neg.
8.	1%	neg.	1%	neg.
9.	1%	neg.	1%	neg.

* TA Telepaque Administration.

tion of telepaque: (b) two weeks following the twenty-our hour evaluation.

Discussion

The results shown in Table I indicate that nine of the nineteen patients show an increase in bromsulphalein retention twenty-four hours after the administration of telepaque, and a decrease in dye retention at the two week interval. The values for the comparative twenty-four hour determination in Table II likewise show an increase in five of the nine subjects. However, these findings are still within normal limits.[4]

The apparent abnormal value of the twenty-four hour specimen of patient #4 was the result of an introduced error, namely hemolysis. Attempts to clear the serum proved unsuccessful. The series of tests for patients #2, 12, and 19 were unable to be completed because of surgical intervention.

Values for the cephalin-cholesterol flocculation tests listed in Tables I and II indicate no significant changes.

Summary

1. A study of bromsulphalein retention and parallel cephalin cholesterol flocculation tests was made before and after the administration of telepaque.
2. Nineteen patients referred for cholecystographic studies and nine student nurses were used in this study.

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THE BIOCHEMICAL INVESTIGATION OF HYALINE MEMBRANE DISEASE*

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When a research project is initiated only the most optimistic of investigators is prepared to forecast the final scope of the work. This paper is presented as an example of a line of enquiry which started with histopathology and finished up in biochemistry—or rather, at present rests in biochemistry. The next stage appears likely to involve us in a mixture of neurophysiology, electronics and steroid chemistry.

The presenting problem is essentially a clinical entity. A large proportion of premature infants who survive their too-early arrival in the world succumb to a condition usually called hyaline membrane disease. They appear reasonably well for the first twenty-four hours of life, but then become cyanosed and despite the use of oxygen, etc., death ensues. Sections of the lungs of these cases show that the alveoli are lined with a layer of hyaline material, forming an all too effective barrier to gaseous exchange. The main questions raised are, firstly, the chemical composition of the membrane, and secondly, the processes leading to its formation.

The first approach to the problem was histochemical. Frozen sections of lung were stained by the Lepehne-Pickworth[1] technique—originally intended for demonstrating the capillary network in brain. This is basically the benzidine-peroxide method for detecting blood, and in the original usage it demonstrates the capillaries by staining very intensely the red blood cells contained therein. It was found that the technique also stained the hyaline membrane very deeply. Further sections were treated with the so-called "Nadi"[2] reagents—alpha naphthol and dimethylparaphenylenediamine. This technique is supposed to be specific for the demonstration of cytochrome and the positive reaction obtained from the membrane at first led us to postulate that the membrane contained the cytochrome system in large amount. But subsequent work showed that the reaction was by no means specific. In order to determine whether any heavy metals were present—as complexes—a series of microchemical tests were made, and the only metal present in any quantity was iron, as shown by positive reaction with dithizone in the presence of reagents—such as citrate—which prevented reactions with other heavy metals. Thus it was felt that the membrane

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contained iron, probably in the form of iron-porphyrin compounds and possibly peroxidase enzymes.

Pulmonary oedema fluid gave similar reactions with the Lepehne-Pickworth and Nadi reagents, as did the cells lining the bronchioles. Thus it seemed probable that the membrane was formed by drying of oedema fluid and its subsequent "plastering" on the alveolar walls. The identity of the main iron-containing compound of the membrane as hemoglobin was determined by converting the substance to a hemochromogen with alkali and ammonium sulphide and then demonstrating the presence of the hemochromogen by microspectroscopy using a Hartridge reversion spectroscope and the ground glass viewing screen of a Leitz "Ortholux" microscope.

Pulmonary oedema fluid was examined both chemically and electrophoretically and was found to consist, in effect, of diluted plasma containing hemoglobin presumably from lysed red cells.

The finding of hyaline membrane in other conditions than the disease of premature babies—for example, adults with bulbar poliomyelitis, traumatic brain damage, mitral stenosis—suggested that the condition was the end result of a derangement of pulmonary neuro-vascular dynamics. An extensive program of animal experiment, in which hyaline membrane was produced by exposure to high oxygen concentrations or by bilateral vagotomy, with sacrifice of animals at various stages, showed that membrane formation was a terminal phenomenon. Earlier work at a previous laboratory had hinted at a biochemical factor, and wide investigation of the biochemistry of the experimental animals at various stages of oxygen poisoning demonstrated most unusual and almost catastrophic biochemical changes.

Briefly summarized, the progress of the condition was shown by a fall in blood pH, a marked rise in serum potassium, a rise in serum inorganic phosphate, and increased erythrocyte fragility. The unusual feature of blood pH values in severe acidosis region with broadly normal CO_2 combining power values is at present being further investigated. It is worth mentioning that the effects of dehydration—as shown by high hematocrit values—were taken into account when assessing the significance of the raised potassium values.

The importance of the vagi in the process of hyaline membrane formation—as indicated by the consistent production of the condition by bilateral midcervical vagotomy—led us to postulate that the initiating factor was the "confusing" effect of abnormal oxygen/carbon dioxide ratios on chemoreceptors in the lungs, leading to disordered afferent stimuli to the respiratory control centres. Coupled with this there is an unusual kind of heart failure—possibly due to potassium loss from the myofibrils

—associated with oedema of the lungs. The production of hyaline membrane by vagotomy is believed to arise from the alteration or loss of all afferent stimuli from the chemoreceptors in the lungs, and certain observations in the experimental animals suggest that an unbalanced action of the sympathetic innervation may be responsible for the bronchial emphysema and pulmonary oedema; the emphysema-induced anoxia leads to heart failure. The escape of plasma and red cells into the alveoli and subsequent drying leads to hyaline membrane formation.

From these findings we are led to postulate that the vagus and sympathetic nervous system have a profound controlling influence on electrolyte and pH balance in the blood, and possibly in such tissues as muscle.

From a technical point of view the main difficulties were:—

i. Obtaining blood from rabbit ear veins in the later stages of the artificially-produced disease, when the slightest struggling on the part of the animal—or even fright alone—caused immediate death from heart failure. Several of our ECG recordings were terminated in this manner; the hearts of the animals had an easily detectable "gallop" rhythm.

ii. Heparin was used as anti-coagulant for blood samples, but we found that the commercial preparations contained enough phosphate to give errors in inorganic phosphate determinations.

iii. Flame photometry for sodium and potassium was straightforward for serum and plasma; but on extracts of heart and pectoral muscle—done in endeavouring to trace the source of the raised serum potassium values—special standards had to be carefully made to correct for the interference of sodium on potassium emission. Similarly, when doing flame photometry of urine for sodium and potassium, the very wide range of results made special standards essential. (Sodium 1.35 to 155 mgm per 100 ml; Potassium 303 to 905 mgm per 100 ml.) It may be of interest to mention the apparent normal serum potassium range for rabbits. About half the values obtained were in the range 13.0 to 14.0 mgm per 100 ml; the rest ranged up to 18.0 mgm per 100 ml.

iv. Red cell potassium values were obtained from hard-packed Wintrobe tube deposits from the hematocrit determinations. The very viscous nature of the red cell mass made accurate measurement difficult. Finally, a batch of 0.1 ml micro-pipettes was carefully checked by mercury calibration. Provided that complete laking was achieved, results were reproducible to within the experimental error of the instrument used—the EEL Flame Photometer—which had been shown by previous estimations to be of the order of plus or minus 1%.

v. The lack of a Waring blendor entailed a laborious process of hand cutting and drying in determining the muscle sodium and potassium. Some doubts were naturally felt about the accu-

racy of such a crude procedure; but duplicate experiments and comparisons with the rather scanty data in the literature showed that the values obtained were reproducible to within the instrument error and tallied very well with the work of others. We feel that the method, while possibly leaving something to be desired, was none the less adequate for the purpose employed.

The work is still in progress. The exact nature of the acidosis, apparently the result of a nervous system malfunction leading to respiratory anoxia, has yet to be determined. The nervous mechanism which responds to high oxygen/low carbon dioxide conditions by apparently initiating profound biochemical changes has yet to be investigated; but here even the methods of enquiry have yet to be worked out.

In conclusion, this work has shown us that lack of expensive equipment, shortage of time and even lack of specialized technical knowledge need not prevent people with a little common sense and power of application and improvisation from attempting to unravel a complex problem. The only insoluble problem is the one you are afraid to tackle.

I wish to express my indebtedness to Dr. M. J. Lynch for permission to produce this paper, to the Sisters of St. Joseph whose generous help made it possible for me to deliver it at the First North American Conference of Medical Technologists, and to my assistant Miss Gail Querney who often carried more than her share of the routine biochemistry of our busy laboratory to enable me to proceed with the work outlined in this paper.

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SOME ASPECTS IN THE BIOCHEMICAL METHOD FOR HEPARIN ASSAY: A MODIFIED METHOD FOR THE METACHROMATIC ASSAY OF HEPARIN*

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Heparin has become relatively well established as a therapeutic agent in atherosclerosis. In addition to its anticoagulant activity, Anderson[1] has shown that heparin has also the ability to perform as a lipemic clearing factor. This function of heparin in lipoprotein metabolism has presented the necessity for an accurate method of determining heparin levels in the blood. Jacques[2] and Gibson[3] have both presented methods in which heparin is isolated and then measured by its metachromatic activity with Azure A and Toluidene Blue O respectively. Freeman[4] has recently published a method in which the isolation technique is similar to that of Gibson but the measurement of heparin is accomplished by its biologic activity. The methods of both Gibson and Freeman have been employed in this laboratory for comparison.

The purpose of this paper is to relate the experiences and observations of the writer in the assaying of heparin and to present certain modifications to improve the accuracy of the determination. The method of Gibson was found to be more practical, economical and adaptable to the routine laboratory and has therefore been used as a basic method. It is the modified method that is given.

METHOD

Heparin is precipitated from citrated plasma with n-octylamine HCl. The precipitate is hydrolyzed with NaOH and reprecipitated with zinc sulphate. After extraction with phosphate buffer, toluidene blue is added and the resulting precipitate extracted with alkaline alcohol. The solution is then read photometrically.

Reagents

M/15 Phosphate buffer pH 6.1

n-octylamine HCl. (2ml n-octylamine, Matheson, plus 1 ml concentrated HCl. Mix. Dilute to 30 ml with distilled water. Adjust pH to 7.3-7.5)

0.5 N NaOH

0.5 N Zinc Sulphate

0.9% Saline

M/10 Phosphate buffer pH 7.8

Toluidene Blue O solution, 100 mg in 100 ml distilled water. Dilute 1:4 with distilled water and filter before using.

Ethyl Alcohol

N/10 NaOH

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

Isolation

2 ml plasma (citrated or axalated) are diluted with 3 ml M/15 phosphate buffer pH 6.1. Mix by inversion. 2 ml of n-octylamine HCl are added with immediate mixing and the tube centrifuged as soon as flocculation begins. If there is no flocculation in five minutes, the tube may be warmed to 40°C for an additional five minutes. The tube is then centrifuged. Decant the supernatant into another tube and immediately add 8 ml distilled water to the precipitate. Shake vigorously until the precipitate is thoroughly washed by suspension. Let stand. To the supernatant, add an additional 2 ml of octylamine and repeat the flocculation, centrifugation, decantation and washing. *Continue the addition to subsequest supernatants until there is no further precipitation.* (Two to four precipitations are usually necessary.) Centrifuge the tubes containing the washed precipitates. Decant washings into other tubes and add 2 ml octylamine to each, following the same procedure described above until no further reaction with octylamine takes place.

Hydrolysis

As soon as all the precipitates have been thoroughly washed, add 1 ml 0.5 N NaOH to each. Place tubes in a water bath at 70°C and agitate from time to time until the precipitate is dissolved. (This can take from 5-30 minutes.) Add 10 ml normal saline and mix by inversion. Heat 10 minutes longer. Remove from water bath and cool to room temperature.

Reprecipitation and Extraction

Add one ml zinc sulphate, mix by inversion and allow to stand undisturbed for thirty minutes. Centrifuge. Decant supernatant into another tube. Add 1 ml zinc sulphate and allow to stand thirty minutes. Centrifuge. To the precipitates, which contain the heparin, add 5 ml distilled water and 5 ml M/10 phosphate buffer pH 7.8. Shake vigorously to resuspend the precipitate and place in water bath at 70°C for six hours. Centrifuge while warm and drain the supernatant into another tube. The herapin is now contained in the supernatant. Add an additional portion of water and phosphate buffer to the precipitate and resuspend. Reheat at 70°C for four hours or longer and again centrifuge and decant the supernatant into a fresh tube. Repeat extraction once more.

To each of the supernatants add one ml toluidene blue. (A blank is prepared with water-buffer mixture and 1 ml toluidene blue.) Occasionally the heparin content is sufficiently high to show discoloration immediately upon the addition of the dye. In such a case, add an additional 1 ml of toluidene blue. Allow the tubes to stand over night. Centrifuge. The heparin is bound to the dye in the precipitate. Decant the supernatant carefully and add 5 ml distilled water to the precipitate. Shake. Centrifuge and discard washing. To the precipitate add 2 ml N/10 NaOH and

8 ml of alcohol. Let stand 25 minutes. Centrifuge. Carefully decant into a cuvette. Read at 530 mu with the blank at 100% transmittancy. (Very concentrated samples should be diluted with 10 ml portions of alkaline alcohol and the readings multiplied by the dilution.)

A calibration curve is prepared by using 0.01, 0.02, 0.04 and 0.05 mg of heparin sodium as a standard. The heparin is precipitated as above.

DISCUSSION

The method given above is essentially that of Gibson, the main difference being the repeated precipitations with octylamine, washings and extractions. Both Freeman and Gibson use practically identical procedures through the hydroxide hydrolysis and, up to that point, the discussion would apply to both methods.

Repeated test on the same plasma gave such varied results in this laboratory that further investigation of the methods of Gibson and of Freeman was found necessary. Different batches of octylamine reacted differently to the concentrated HCl addition. Some did not even form the hydrochloride. Assays run on the same plasma gave widely different results when precipitated with different octylamines. Table I shows both of these variations.

TABLE I

Heparin Determinations Using Same Plasma With Different Octylamines. (Using Method of Gibson)

Octylamine	Reaction to HCl Addition	mg/5 ml Plasma
No. 1	Clear, colorless solution	.005
2	No hydrochloride formed	....
3	No hydrochloride formed	....
4	Clear, colorless solution	.017
5	Clear, tainted solution	.020
6	Clear, tainted solution	.003
7	Cloudy, tainted solution	.008
8	Clear, colorless solution	.013

It was felt that recovery of the poorer precipitated plasmas would be found in the supernatants which were being discarded after the original octylamine precipitations. The single washings prescribed by Gibson and by Freeman were also tested and both proved to contain considerably more heparin than the original precipitation. This led to reprecipitations of the original supernatants and their washings until there was no further reaction with octylamine, second additions of zinc sulphate to the hydrolyzed tubes and repeated extractions of the zinc complex with phosphate buffer until all results were negative.

One sample by this method could require from 36 to 48 or more readings, making such a procedure rather impractical for

any routine analysis. Completion of the test, however, gives highly accurate and reproducible results. Table II gives a comparison of three assays run on the same plasma using the methods of Gibson, of Freeman and the method described above. Three different octylamines were used. The remarkability of the accuracy as well as the difference in amount of heparin found by the three methods is obvious. Complete analysis would yield 8.1 mg per 100 ml of plasma. Gibson reports a normal range from 0.02-0.54 mg% and Freeman finds 0.05-0.20 mg%.

TABLE II

Comparison of Two Methods With Modified Method on Two ML Portions of the Same Plasma

Plasma Tube	Octyl-amine	Test per Gibson	Test per Freeman	Modified Method			
				A*	B†	C‡	Total
No. 1	No. 1	.003mg	.010mg	.026mg	.078mg	.057mg	.161mg
2	2	.009	.004	.042	.072	.050	.164
3	3	.003	.013	.038	.069	.062	.169

* From repeated Zn pptn's and phosphate extractions.
† From repeated octylamine precipitations of supernatants of original.
‡ From precipitations of washings.

The immediate question following is that of whether or not the material obtained is heparin. Octylamine is a heparin-specific precipitant[5] and it may be assumed that the precipitating material is heparin or heparinoid in nature. The material recovered from the octylamine supernatants and washings all show anticoagulant activity as measured by the Freeman method. (Table III.) This has been confirmed by another investigator using the Freeman method.[6]

TABLE III

Comparison of Metachromatic Activity and Anticoagulant Activity of Washings and Supernatants

Content	Original ppt. With Octyl.		1st Wash Original		Supernatant of Original		2nd Wash Original		Second Supernat.	
	G	F	G	F	G	F	G	F	G	F
Plasma No. 1...	.008	.010	.018	.015	.004	.004	.034	.028	.008	.004
Plasma No. 2...	.012	.009	.024	.015 mg%	.000	.003	.018	.031	.006	.002

Further comparison of the Freeman and the given method for anticoagulant activity at various stages is not possible due to the variations in methods. This is unfortunate since as much as 40-50% of the total yield is often recovered by additional phosphate buffer extractions. Since the Freeman method does not employ the buffer extraction, this step cannot be compared for

anticoagulant activity. The procedure of Gibson, however, with one phosphate extraction, shows anticoagulant checks.[4] Since no reagent other than that already used in the Gibson method is introduced in the repeated extractions, it may follow that the secondary extractions will also give biologic activity. To substantiate this assumption, activity with protamine sulphate has been used to demonstrate anticoagulation. An assay was run in triplicate. Two samples were used for reliability checks when toluidene blue is added on different days. The third sample had 0.5 ml of 10% protamine sulphate added to all tubes before toluidene blue addition, allowed to stand overnight for complete removal of heparin and the dye added the following day. Table IV shows results obtained.

TABLE IV

Experiment Showing Activity of Heparin With Protamine

Plasma	From Repeated Octyl. pptns.	From Repeated Zn pptns.	From Repeated Extractions	Total
No. 1 Tube 1	.028mg/2ml	.022mg/2ml	.112mg/2ml	.162mg/2ml
*2	.029	.020	.115	174
**3	.000	.0005	.0005	.001
No 2 Tube 1	.044	.026	.078	.148
*2	.046	.039	.057	.142
**3	.000	.0005	.000	.001

* Toluidene blue added next day.
** Protamine added first day; toluidene blue added next day.

Further proof of the presence of heparin is the recovery experiment on plasma to which heparin had been added. This was an in vitro experiment. The results were further substantiated by an in vivo experiment shown on the same table. (Table V)

TABLE V

Determinations on In Vivo and in Vitro Heparinized Blood Using the Modified Method

	In Vivo		In Vitro
Plasma before heparin	9.2mg %	Plasma before heparin	8.8mg %
Plasma 1 hour after 100 mg heparin I.M.	30.4mg%	Plasma after 50 mg% added	56.4mg%

Mucoprotein is about the only other known substance in blood that is heparin-like in nature. Table VI shows that there is no correlation between the serum mucoprotein values and the plasma heparin content.

One problem unsolved, however, is the irregularity of the stage of appearance of the heparin. Most samples show the highest percentage of heparin after the second phosphate extraction even though the first was relatively negative. Since only

TABLE VI
Individual Determinations Comparing Heparin Assays and Mucoprotein Content

PATIENT	Heparin mg %	Mucoprotein mg %
No. 1	9.8	40
2	7.9	40
3	5.1	00
4	9.7	00
5	10.1	110
6	12.8	00
7	9.7	200
8	11.4	75
9	8.9	230
10	8.3	00

additional buffer was added and careful pHing showed no change, the results might suggest the removal of some interfering substance by the first extraction. This phenomenon might also suggest the presence of different kinds of heparin—that is, free versus protein bound heparin or isometric heparin with different arrangements of the OSO_3—radical. Another frequent high heparin stage is at the subsequent octylamine precipitations of the original tube. This may be due to variations in the octylamines. This phase requires considerably more research, however, before any conclusions can be drawn.

The determination works as well on hemolyzed plasma as on normal plasma. One hemolyzed and heparinized sample of plasma was mistaken for non-heparinized plasma and led to testing of whole blood for increased amounts. Attempts to carry the test through on whole blood thoroughly hemolyzed, treated with perchloric acid and other means have given lower results than plasma assays but investigations are still being carried on in this direction.

SUMMARY

A modified method for the determination of heparin is described. This method demonstrates the presence of much larger amounts of heparin in normal blood than ever previously reported. This will be important to those who are employing heparin therapeutically or are attempting to use heparin blood levels for diagnostic purposes. This modification, when carried through to completion, offers highly reproducible and accurate results. Recoveries of in vivo and in vitro heparin additions are good. That the determination measures heparin (or heparinoids) is further substantiated by the fact that it is an octylamine precipitable, thermostable substance showing both metachromatic and anticoagulant activity. This is all characteristic of heparin. Determinations on normal patients show a mean value of 9.8 mg% with a range of 4.8—13.8 mg% found to date.

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AMONG THE NEW BOOKS

LYMPHATICS, LYMPH AND LYMPHOID TISSUE. By Joseph Mendel Yoffey, D.Sc., M.D., F.R.C.S., Professor of Anatomy, University of Bristol, England, and Frederick Colin Courtice, M.A., D.Phil., D.Sc., F.R.A.C.S. (Hon), Director Kanematsu Memorial Institute of Pathology, Sydney Hospital, Sydney, Australia. 1956. The Commonwealth Fund, Harvard University Press, Cambridge, Mass. 510 pages, 99 illustrations, charts, 55 tables. $10.00

Primarily a reference for the research worker in medicine, this volume will, nevertheless, prove valuable to the hematologist and histology technician, as well as to the pathologist and radiologist.

With a detailed description of the anatomical and physical aspects of the lymphatic system in mammals, the authors indicate its clinical significance. The chemical composition, physical characteristics, cell content, flow and pressure of lymph are all included, together with the significance of the lymphocyte. The chapter devoted to the study of the lymphocyte is the most practical for the medical technologist, especially in the section devoted to the lymphocyte as it relates to virus diseases.

ULTRAMICRO METHODS: FOR CLINICAL LABORATORIES. By Edwin M. Knights, Jr., M.D., Associate Pathologist, Director of Clinical Pathology and Blood Bank; Roderick P. MacDonald, Ph.D., Director of Clinical Chemistry and Research Advisor; Joan Ploompuu, Chief, Division of Ultra-Micro Chemistry and Research Associate. All of Harper Hospital, Detroit, Michigan. Grume & Stratton, New York-London. 1957. 128 pages, 18 illustrations. Tables. $4.75.

With the increasing use of micro and ultra-micro methods in the clinical laboratory, a collection of these techniques is timely and needed. This volume describes the set up of such a division in the laboratory, and warns of the necessity for comparison with macro procedures, and the continuous use of controls to insure accuracy.

Beside the usual chemical procedures, electrochromatography, microhematocrits, C-Reactive protein, and micro floccuation tests for syphilis are described.

MICRO-ANALYSIS IN MEDICAL BIOCHEMISTRY. 3rd Edition. By E. J. King, M.A., Ph.D., D.Sc., F.R.I.C., Professor of Chemical Pathology in the University of London, Post graduate Medical School; and I. D. P. Wootton, Ph.D., M.A., M.B., B. Chir., F.R.I.C., Lecturer in Chemical Pathology at the Postgraduate Medical School, London. Grune & Stratton, New York. 1956. 292 pages. 25 illustrations. 18 tables. $4.00.

In this edition the importance of accuracy and quality control is brought out. With each technique is a brief statement of principle as well as a description of the method. In the preface the author states that "emphasis has been maintained on the micro aspects of analysis, without going into the field of what might be called the 'ultra-micro.'"

Procedures for feces, urine, gastric contents, and cerebrospinal fluid are described as well as those primarily for chemistry of blood. This and the volume reviewed above might be described as supplementary to each other.

(Continued on Page 180)

RADIOACTIVE IODINE AND PROTEIN BOUND IODINE VS. BASAL METABOLIC RATE AS A MEASURE OF THYROID FUNCTION*

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Prior to a few years ago the only reliable means a physician had of measuring the activity of the thyroid gland clinically was to do a basal metabolic rate determination (B.M.R.) on the patient. This was a fair indication of thyroid function, but it is subject to many errors, both technical and physiological. Now, however, the doctor has two new techniques which he may use to help in the diagnosis of thyroid disease; namely, measurement of radioactive iodine (I^{131}) in the thyroid gland and chemical determination of the protein bound iodine (P.B.I.) in the blood serum. These new techniques have proved more reliable and accurate than the older technique of B.M.R. determination for the measurement of thyroid function.

In a test carried out by the U. S. Naval Hospital and U. S. Naval Medical School, Bethesda, Maryland, on fifty euthyroid and twenty-five hyperthyroid patients the following results were obtained:[1] After oral administration of ten microcuries of $I,^{131}$ normal (euthyroid) patients showed a concentration of between 5% and 45% radioactive iodine (I^{131}) in the thyroid gland in 97% of the cases; whereas, in hyperthyroid patients, over 94% of the cases showed a concentration of between 50% and 85% of $I.^{131}$ Also, in determinations of blood serum P.B.I., the normal subjects all showed between 3.5 and 8.0 gamma of P.B.I. per 100 cc of serum, while the hyperthyroid patients all showed between 8.0 and 16 or even more gamma per 100 cc of serum. Carefully controlled conditions were maintained in all these tests, such as making sure patients were in fasting condition and had received no iodine-containing medications, such as desiccated thyroid, or iodized oils which are used in roentgenographic diagnosis.

It is agreed, that a single determination of basal metabolic rate has limited value in the diagnosis of thyroid function. Also, the expense and facilities, as well as the technical help, needed for B.M.R. determinations could be more advantageously used for the more accurate P.B.I. and I^{131} tests. This is concluded from a comparison of B.M.R. and I^{131} tests done on fifty euthyroid patients.[1] It was found that 84% of these patients had a I^{131} uptake between 10% and 45% in twenty-four hours, which is considered the normal range. The other 16% were only slightly below 10% or slightly above 45%. When basal metabolic rate determinations were done on these same patients (fifty euthyroid

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

patients) it was found that only 60% were in the normal range and had basal metabolic rates which varied from minus 30 to plus 40 per cent.[1] Silver[2] and others have stated about basal metabolic rates: a B.M.R. is not only "a poor adjunct to clinical judgment, but one which is affected by many factors other than thyroid hormone."

A protein bound iodine test is of value in diagnosis of thyroid function because the presence of iodine in the blood fluctuates with changes in thyroid function and this iodine is the iodine of the circulating thyroid hormone. It has been found by Dr. Douglas S. Riggs that there is no strict correlation between the P.B.I. of the blood serum and basal metabolic rate, due to the fact that many extrathyroid factors may influence the B.M.R. He also found that when he gave patients thiouracil the serum iodine level fell to subnormal values when the B.M.R. was normal or above normal.[3] Another way in which the P.B.I. is superior to the B.M.R. as far as thyroid function is concerned is that in a blood determination the patient's attitude or emotional state does not alter the results, whereas, in the B.M.R. determination the patient's emotional condition has a great deal to do with the results obtained. Furthermore, it may prove difficult to obtain satisfactory B.M.R. determinations on patients with respiratory disorders.

The major drawback in doing P.B.I.'s is that one must be absolutely sure that the patient has had no therapy or treatment with any iodine-containing compounds such as are used in radiological work. If the patient has had this sort of therapy it will show up in a P.B.I. determination many months after such therapy has been discontinued.

Radioactive iodine determinations have been very useful in the diagnosis of thyroid function. In this determination radioactive isotopes of iodine are given and then the amount of this substance is measured both in urinary excretion and in uptake by the thyroid gland to determine how much of a standard dose of iodine has been utilized by the thyroid gland. By this means the isotope can be distinguished from iodine already present in the body.[3] A low urinary excretion of the radioactive iodine which has been given to the patient indicates that the thyroid gland is able to retain the iodine, and if the retention is above a certain normal level it can usually be concluded that the patient is in a hypermetabolic condition. Thus, hypermetabolism due to other causes such as leukemia, lymphoma, cardiac failure, hypertensive vascular disease, etc., can be ruled out.[3]

Many criteria are used to measure thyroid function. For example, P.B.I., B.M.R., radioactive iodine tracers, cholesterol level in the blood and creatinine tolerance tests have been used in determining thyroid function. Actually all of these tests have

some value for measuring thyroid activity: "B.M.R. is an index of the total impact of thyroid hormone on all its end-organs, P.B.I. is an expression of thyroid hormone concentration in the blood and radioactive iodine is a measurement of the thyroid gland's avidity for that element."[4] However, I consider the latter two determinations the most accurate in diagnosis of thyroid malfunction due to the fact that the B.M.R. determination has many more sources of error, both from a technical and from a physiological standpoint. P.B.I. determinations are probably more complicated and require more skill and expense than basal metabolic rate determinations, but considering the fact that many more cases of thyroid disease can be picked up by this method with a high degree of accuracy this test should become part of the routine examinations of every clinical laboratory and should be run in conjunction with B.M.R.'s, cholesterols, creatinine tolerances, and radioactive tracer studies.

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AMONG THE NEW BOOKS

BLOOD AND BONE MARROW PATTERNS. By G. D. Talbott, M.D., formerly Chief of Medicine, and Elmer R. Hunsicker, B.S., formerly Chief of Laboratories, both of 2750th Hospital, and Respiration Section, Aero Medical Laboratories, Wright-Patterson Air Force Base, Dayton, Ohio, and Jonah Li, M.D., University of California Medical Center, San Francisco, California. Grune & Stratton, New York and London, 1957. 59 pages, 145 color micro-photographs. $12.00.

A teaching atlas whose format is designed for convenient reference at the microscope, presents a series of 23 blood cell patterns in various abnormal conditions. There are five photographs relating to each of the anemias, leukemias, lymphomas, and other unrelated abnormalities described. Where bone marrow patterns might prove of value in diagnosis, they have been included with those from peripheral blood. Each photograph is briefly and concisely described. These legends comprise the text. 30 microphotographs of the red cell series, myelocyes, eosinophils, basophils, monocytes, megakaryocytes, plasma cells, and lymphocytes, with the same cryptic descriptions, form the introductory portion of this excellent training aid.

DEATH OF A MAN. By Lael Tucker Wertenbaker. Random House, New York, N. Y., 1957. 181 pages. $3.50.

Not a volume of laboratory techniques, but one for you—and me—to read—and appreciate. With cancer a leading cause of death in our ranks this story comes close to each of us. The author shows the thoughts and feelings of herself—and her husband—who wrote about the first hours—when he learned of his illness. There is a courage shown here—a love of living—and honesty—facing death without regrets. "As a hoper, not a dreamer, he

(Continued on Page 183)

POTENTIAL DANGER TO LABORATORY WORKERS OF THE TRISODIUM PHOSPHATE METHOD IN THE DIAGNOSTIC CULTURE OF TUBERCLE BACILLI

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Although several publications have described the use of trisodium phosphate (T.S.P.) for the diagnostic culture of tubercle bacilli,[1, 2, 3] we have not encountered any warning of its potential danger to laboratory workers. This danger is due to two factors:

(1) T.S.P. is a detergent, and if it comes in contact with the top of a tube or bottle, has an unusual ability to work its way between the cork or screw cap and the glass. If the material being treated contains tubercle bacilli, the T.S.P. which thus creeps up carries tubercle bacilli with it. This would probably have no significance but for the second factor involved:

(2) Due to the incubation of 24 hours or longer, any T.S.P. which has seeped up past the cork, or the liner of the screw cap, dries to a friable white crust. On removing the cork or cap, this contaminated material crumbles, often falling onto the operator's hands: it is possible that some of this white crust, as it crumbles, forms a powder fine enough to be airborne, and hence especially infectious.

Once these factors are understood, the technique used can be modified so as to avoid the danger to laboratory workers which is indicated above. Some suitable modifications have been made by workers who perceived the need for it, although they did not publish any note regarding the dangers they were thus avoiding.

The following procedures are believed to be satisfactory and overcome the potential dangers of the T.S.P. method. Equally satisfactory methods may be devised by other workers.

(1) Add the T.S.P. solution to the specimen, shake to obtain a uniform suspension, preferably in a mechanical shaker, and pour into a wide centrifuge tube, taking care to avoid contaminating the top of this tube. Flame the top of the tube, and replace the cover, taking care that the liquid does not come in contact with the cork or cap used. The centrifuge tube is then incubated 24 hours or longer, centrifuged, and the sediment neutralized and cultured.

(2) Supply patients with a 16 or 20 ounce wide mouth bottle, containing one ounce of T.S.P. solution. A mark on the bottle indicates the two ounce level. Patients are asked to add sputum until this level is reached. With this size of bottle, handled with reasonable care, there is little danger of the cork or cap becoming contaminated. On returning to the laboratory, the bottle is not shaken, or at most only gently rotated, so that the cork or cap is not contaminated. After incubation for 24 hours or longer,

part of the supernatant is discarded, and the lower part is transferred to a centrifuge tube, centrifuged and the sediment treated in the usual way.

(3) Tarshis & Lewis[4] use a 6 x 1 inch screw cap tube, greasing the rim of the tube with vaseline. In this tube the sputum and T.S.P. solution are placed, shaken, incubated, and centrifuged. The authors state that the "creeping" of the T.S.P. solution past the liner of the cap is prevented by greasing the rim of the tube with vaseline.

(4) If the sputum or other material is shaken with T.S.P. solution, and then incubated at 37° C in a water bath provided with a well fitting cover, "creeping" of the mixture may occur, but the humidity will be sufficiently high to prevent this material from drying out to a crust around the stopper. Thus the danger to the technologist is minimized or overcome. If bark corks are used, they will often come out of the tubes during this incubation, unless held in place; this is due to the slippery, detergent nature of T.S.P. solution. There would be no trouble with screw cap tubes.

In this laboratory, after giving the T.S.P. method a trial, we have gone back to the use of sodium hydroxide with mechanical shaking. We feel that small errors of technique or apparatus may at times lead to danger to our staff, as indicated earlier in this article. Also, when using T.S.P. without mechanical shaking, essentially as method "two" above, our microscopic findings were much lower than by the sodium hydroxide method with mechanical shaking

The diagnostic culture of tubercle bacilli is not without danger to laboratory workers, regardless of the method employed. It is our opinion that every hospital and sanatorium laboratory should be provided with a suitable cabinet in which most of the manipulative work can be done, and in which slides made with infectious material can be allowed to dry. Such a cabinet would have: (1) A sheet of glass between the worker's face and the material he is handling; a space of about 10 inches below this sheet of glass and the bench top allows handling of samples. (2) A current of air drawn through the cabinet, so that any accidental spray or nebulized material is carried away from the worker.[5]

Summary—The trisodium phosphate method for the diagnostic culture of tubercle bacilli can be a health hazard to the laboratory worker, unless the technique is suitably modified to overcome this danger.

Acknowledgment—We are indebted to Dr. A. R. Armstrong, Mountain Sanatorium, Hamilton, Ontario, and Mr. R. Karn, Provincial Health Laboratory, Woodstock,

Ontario, for two of the methods outlined in this article.

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AMONG THE NEW BOOKS

(Continued from Page 180)

left his world, having found it imperfect and good, hopeful for it, hoping that the best he had been would live after him." There's a kind of comfort in this "Death of a Man." Read it.

A LAB-COAT POCKETFUL OF SAFETY. Compact, pocket-size, fully illustrated, the newest edition of the Fisher *Manual of Laboratory Safety* covers accident prevention, first aid, fire prevention, and safety equipment, and concludes with a safety bibliography. It also has a section on handling radioactive materials, as well as the use of isolation units for hazardous microbiological and clinical procedures.

The *Manual* is published by Fisher Scientific as a service to laboratories and clinics everywhere; free copies are available from Fisher Scientific Company, 455 Fisher Bldg., Pittsburgh 19, Pennsylvania.

ALSO RECEIVED:

MANUAL OF RADIATION THERAPY. By K. Wilhelm Stenstrom, Ph.D., Professor of Geophysics, Director, Division of Radiation Therapy, University of Minnesota Medical School. Charles C. Thomas, Springfield, Ill., 1957. 94 pages. Tables, charts. $4.50.

INDUSTRIAL DEAFNESS: Hearing, Testing and Noise Measurement. By Joseph Sataloff, M.D., D.Sc., Ass't. Professor of Otology, Jefferson Medical College. The Blakiston Division, McGraw-Hill Book Company, Inc., N. Y., 1957. 333 pages. Illustrations, charts and tables. $8.00.

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HEEDLESS HORSEPOWER. The Travelers Insurance Companies, Hartford, Connecticut, 1957. Distributed free in the interest of street and highway safety. Order from the Companies or their representatives.

HOME HEALTH EMERGENCIES—A Guide to Home Nursing and First Aid in Family Health Emergencies. The Equitable Life Assurance Society of the United States, 393 Seventh Ave., New York, N. Y., 1957.

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BIOLOGICAL STAINS AND RELATED PRODUCTS FOR MICROTECHNIC. By Hartman-Leddon Company, 5821 Market Street, Philadelphia 39, Pennsylvania. The brochure features complete lists of Biological Stains in crystalline as well as solution form along with listing of the products known as Harleco Parstains and finally a section devoted to Histological and Histochemical Reagents. Copies free upon request.

A MEDICAL TECHNOLOGY TRAINING PROGRAM STUDY*

RUTH F. HOVDE, M.S.,[1] MT (ASCP)

The primary purpose of this presentation is to report the development and activities of the Course in Medical Technology at the University of Minnesota. Furthermore it seems appropriate to present general information about this new profession of medical technology in order to promote better understanding and interest in this field of medical science.

Thirty-five years ago the Board of Regents of the University of Minnesota awarded the first degree of Bachelor of Science with the designation of specialization in Medical Technology. Because of the difficulty in finding and piecing together the meager historical facts about the establishment of this course, it seems appropriate to record some of the known events.

Dr. Wesley Spink,[1] in speaking about the program here, referred to the "birth of a concept" and the initial planning for a program in Medical Technology as the first of three eras in the development of this curriculum at the University of Minnesota. During this period a committee composed of Dr. S. Marx White, Dr. Wilfred Larson, and Dr. Richard Olding Beard, who served as chairman, directed the course, setting the pattern of high standards of performance and the requirement of a four-year-degree program which have remained to this day. A degree program in medical technology was a complete innovation at that time. Concerning this revolutionary idea Dr. Beard[2] in 1923 wrote:

> "The apprenticeship plan has been the cheapest and the earliest plan and it is still followed, in most institutions, as the only surviving relic in any calling of our educational past. Apprenticeship has been practiced under the name training school but in fact and in operation the school has been more like a shop in which novitiates have served a term of almost exclusively practical experience, determined as to its length more often by the demands of the hospital service than by the content of teaching or of the needs of the student."

It is not surprising, then, that under his leadership the original requirements for admission to this course were the same as for admission to the College of Science, Literature, and the Arts and that the value of a combined liberal arts and professional education was recognized and established as the basic plan for this program.

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* This is an address given at the Staff Meeting of the University of Minnesota Hospitals on January 11, 1957.
Reprinted from the Medical Bulletin of the University of Minnesota Hospitals, Vol. XXVIII, No. 6, January 15, 1957.

[1] Assistant Professor in Medical Technology.

The first course bulletin[8] was published on March 10, 1922 with the title, *Courses in Medical Technology for Clinical and Laboratory Technicians.* It contained the following announcement: "The demand for clinical and laboratory technicians trained in the principles and techniques of the medical sciences is increasing. The technician so trained is an aide to the physician, the surgeon, the medical specialist, the group clinic, the hospital, or the teaching and research laboratory. The vocation is one that offers satisfactory objectives, a large measure of usefulness and fair compensation." The course requirements of the first two years in the College of Science, Literature, and the Arts included credits in mathematics, chemistry, English, foreign language, and animal biology.

Four sequences were offered at that time to the student in the third and fourth years of the Course in the Medical School: a. sequence leading to service as a clinical technician; b. sequence leading to technical service in pathology, neuropathology, and gynepathology; c. sequence leading to technical service in bacteriology and serology; and d. sequence leading to technical service in anatomy or neurology.

No specific details about the clinical training were mentioned other than this statement: "Students in clinical technology, as soon as they have adequate preparation, will be assigned to practical work, for one or more quarters, in the technical laboratory of either University Hospital, Minneapolis General, or Charles T. Miller Hospital in all of which a wealth of clinical material is available and where expert supervision and training will be given." Evidently no formal clinical training was established, but rather the student in any of the sequences could do whatever practical work could be managed in between classes, often only a part of two or three mornings each week. This situation is not surprising in view of the many formal requirements of the course.

Upon Dr. Beard's retirement in 1925 Dr. William A. O'Brien was appointed chairman of a special committee composed of representatives from the Graduate and the Medical Schools to direct the Course. Under Dr. O'Brien's leadership the second "era of growth" emerged. During this time the curriculum content and the clinical training program were crystallized.

In 1929 the first program of rotation through the various clinical laboratories with definite assignments in each was established, but classes on the campus still took up a large part of each day. By 1932 the clinical year of 12 months was arranged for each student as follows:

General Laboratory (Hematology, Chemistry, and Bacteriology)	4 months
Tissues	2 months

Metabolism and Electrocardiography	1 month
Serology	1 month
*X-ray	4 months

In 1934 a regular system of submitting grades to the Registrar's office was started. Previous to this time whatever number of credits the student needed to complete 180 credits for graduation was submitted. Since this time a minimum of 45 credits in the senior year of clinical training has been required.

In 1936 the first full-time instructor and a budget of $600 a year to be used for teaching purposes were allocated to the Course. Mrs. Gleva Blain Erskine was appointed the first instructor. Mrs. Erskine had come to the University Hospitals in July, 1924, had served as "technician in charge" of the laboratory, and had gradually assumed the additional responsibility of supervising the student program. With this budget then, according to a department report,[4] "sufficient funds were now available for mimeographed examinations, a supply of pipettes and counting chambers with which the students could practice, and textbooks for reference in the laboratory."

In 1939 the third "era of maturity" started with the appointment of Dr. Gerald T. Evans as Director of the Course in Medical Technology and Director of the Laboratory Service of the University of Minnesota Hospitals, in which capacity he serves at the present time. Under his guidance further changes in the curriculum and particularly in the senior clinical year have been made throughout the recent years. The current curriculum includes 2 years in the College of Science, Literature and the Arts and 2 years in the College of Medical Sciences, the second of which consists of 12 consecutive months in a rotating service in the clinical laboratories of the University of Minnesota Hospitals.

The clinical training in the fourth year is dependent on the Laboratory Service of the Hospital. The personnel in both areas contribute materially to both the teaching and the service functions of the clinical laboratories. The first available detailed report of laboratory determinations appeared in the Hospital Report for 1927-28 and indicated that 24,869 tests had been carried out during that academic year. A similar report for 1955-56 showed that 519,005 tests had been performed.

In 1927-28 only ten different chemical determinations were done, while at the present time the chemistry laboratory is prepared to do 63 different procedures. The same situation exists for almost all the laboratory units and creates the necessity of a flexible training program with changing emphasis from time to

* The inclusion of training in X-ray techniques in the fourth year later became an optional offering with the training in laboratory procedures lengthened to the entire 12 months.

time in keeping with the changing demands on the laboratory service.

The Course in Medical Technology here is unique in that the University of Minnesota was one of the first state universities, if not the first, to offer such a program at a degree level. In fact, university training for technical laboratory personnel has only recently been universally accepted. Clinical laboratory work is now beyond mere repetitive mechanical manipulations. Experience has shown that without education, consistent quality of work, let alone advancement in technical skill and knowledge, is impossible. The Course here has always been planned with the objective of teaching our students in such a way that, as graduates, they will be prepared to train other students and will be capable of accepting the responsibility for reliability of work and keeping up with advances in laboratory science. To this end the curriculum includes not only fundamental sciences but also introductory material in pathology and presentation of case material to assist the student in better understanding of the use and value of accurate laboratory tests.

The strength of the Course lies in the excellent cooperation with the other departments in the basic sciences in course offerings for our students and to the interest and encouragement consistently given by Mr. Ray M. Amberg, Administrator of the University Hospitals, and by Dr. H. S. Diehl, Dean of the College of Medical Sciences. The success of the program is dependent upon the efforts of our staff who have willingly accepted increasing responsibilities in teaching and demands for service.

In the 35 years of operation, 1,110 students have been graduated from the Course. In the period, 1922-1926, there were 13 graduates, in 1927-1936, 201 graduates, in 1937-1946, 536 graduates, and in 1947-1956, 360 graduates. Along with the decrease in the number of graduates in the past ten years, there has been an increased demand for more well trained technologists. The situation is further accentuated by the fact that many of the people working in laboratories are inadequately prepared to meet the needs of good laboratory service. The overall decrease in students in this Course specifically appears to be primarily due to a marked decrease in numbers of students transferring here at the third year level to complete requirements for a professional course. Before 1950 the number of transfer students averaged between 20 and 25 students per year. Since 1950, the annual transfer rate has averaged nine students.

The shortage of students in this field is not confined to this school alone but exists as a general situation throughout the country. Nor, in fact, does this situation exist for medical technology alone; there is a general need for more students and

graduates in all allied medical professions. Reasons for this situation are complex. A report of the Council on Medical Education and Hospitals of the American Medical Association[5] in 1954 on the continuing shortage of well trained technical personnel stated: ". . . Although great progress has been made throughout the period of active standardization, it has not been possible to meet all of the needs for well trained technical personnel. Shortages are continuing in all areas and may be related to a number of factors such as the increasing competition with other fields, the mounting cost of education, prolonged periods of training, inadequate economic returns, high attrition rates, insufficient publicity, limited resources for recruitment, and the need for additional training centers in various parts of the country . . ."

The actual need for personnel in this field is difficult to determine with any confidence. In 1953 there were 5,832 hospitals in the United States with a bed capacity of approximately 1,170,000. Estimating one technologist for every 25 patients, 47,000 technologists would be needed for hospitals alone. At that time 22,741 full-time and 3,041 part-time technologists were employed by registered hospitals, 23,000 short of the ideal number.[6] These figures do not take into account the need for technical laboratory personnel in physicians' offices, clinics, and research laboratories.

All allied professional groups both at the local level and at a national level have been trying within the last few years to find solutions for this situation. Efforts are being made along the following lines:

1. Creation of the National Committee on Careers in Medical Technology to provide better information to the public and, through high school counselors and other appropriate agencies, to prospective students about the need for people in this field and about opportunities in this profession as a vocation.
2. Studying and surveying salary levels and working conditions.
3. Conducting critical evaluations of training programs to improve proficiency of graduates.
4. Studying means for scholarship assistance for students in this field.
5. Encouraging use of auxiliary personnel (clerks, aides, dishwashers, etc.) wherever possible.

What effect these efforts will have is difficult to foretell. It is probably safe to assume that in view of the reports regarding predicted increase in college enrollment that the numbers of students in this Course will increase proportionately.

In order to determine the needs for the State of Minnesota, the Minnesota Medical Association and the Minnesota Hospital Association sponsored a statewide survey of physicians in 1951 with the cooperation of the Minnesota Department of Health.

The survey was specifically designed to secure information regarding laboratory and X-ray personnel, services, and facilities.

The returns were tabulated by the Hospital Services Program of the Minnesota Department of Health and reported in November, 1951. In summary the report indicated:

1. A real need not only for more technologists but also for better trained personnel for laboratory work.
2. A need for technicians (sub-professional level) well trained in limited procedures for physicians' offices and in hospitals under supervision.
3. An interest in a visiting technologist consultant program.
4. An interest in short courses of refresher type to improve quality of the work of the personnel presently employed.

The first aspect of the Minnesota survey that involved this department was the request that our staff, together with that of the Minnesota Department of Health, participate in planning and teaching an experimental group of refresher courses under the sponsorship of the Minnesota Hospital Association, the Minnesota State Medical Association, the professional societies of the pathologists and the medical technologists, and the Department of Continuation Medical Education of the University of Minnesota. Seven courses were held in 1952 and 1953 in Little Falls, Mankato, Hibbing, Crookston, and St. Cloud. These courses were designed to assist the laboratory personnel presently working in rural areas to improve service by review and discussions of techniques and methods. These courses were not intended to be training courses for unskilled personnel but rather for those with some training.

In a critical evaluation of this type of an educational endeavor it must be pointed out that many difficulties were encountered. Among them were the following:

1. The people taking the courses presented a great lack of uniformity in fundamental training and experience. Providing appropriate lectures, laboratory exercises, and demonstrations for such a group was difficult.
2. Lectures alone were not successful. Actual laboratory exercises are necessary. This limits the places where such courses can be held and practically makes it mandatory that they be held at teaching institutions where laboratory facilities and equipment are available. A further complication is the provision of sufficient instructors to staff such courses.
3. On the other hand, for many technologists sessions almost have to be held in the evenings at easily accessible locations. Many hospitals in the rural areas have only one technologist who can not be away during the day or for several days at any one time.
4. There is a grave question about the advisability of attempt-

ing to teach in a few hours techniques for which regular, approved training schools require several weeks.

5. There is the danger that these courses may be regarded as an easy, cheap way to train laboratory assistants.

In spite of these difficulties however, the response to the program by the registrants indicating an interest in attending future similar courses was heartening. Likewise the sponsoring groups and the instructors indicated continuing interest and cooperation in future courses. In view of these facts and the need for continuation education programs in this field such activities should perhaps be extended and developed. Although the concept of regional educational programs is not new, the application of this idea to the field of clinical laboratory techniques is unique and has attracted considerable interest.

The second aspect of the Minnesota survey that directly involved this department was the fact that laboratory assistants with thorough training in techniques within limited areas of laboratory work could be effectively used in certain laboratories. Our own observations and experience with the refresher courses in the state as well as with laboratory sessions for physicians who were trying to give good laboratory service with unskilled and poorly trained assistants substantiated this need.

In 1951 a course of study for laboratory aides under the jurisdiction of this department and offered through the General Extension Division of the University of Minnesota was put into operation. The current program includes two phases of training. The first part, 6 months, includes registration on the campus in special classes involving fundamental material in anatomy, chemistry, and bacteriology and special laboratory periods involving instruction and practice in the basic techniques in hematology, urinalysis, blood bank, and chemistry, and in BMR and ECG tests. The second part, 6 months, includes practical experience in these various aspects in the laboratories of the hospitals affiliated with this program.

The hospitals cooperating with the program in the practical experience of the second part of the training include Abbott, Asbury, Deaconess, Doctor's Memorial, Fairview, Mt. Sinai, North Memorial, St. Andrews, Bethesda, St. Luke's hospitals in Minneapolis and St. Paul, and Hibbing General Hospital, Naeve Hospital in Albert Lea, St. Joseph's Hospital in Brainerd, St. Joseph's Hospital in Mankato, Our Lady of Mercy Hospital in Alexandria, Miller Memorial Hospital in Duluth, Virginia Municipal Hospital, and Community Memorial Hospital in New Prague.

Because this course represented such a drastic departure from our previous experience with the professional course, the initial planning was carried out in conjunction with the professional

medical, hospital, and medical technology organizations in the state. Periodic consultations have been held with these groups, and the program has their complete support. Dr. Ellis Benson[7] has stated: "(The course) represents an attempt to provide satisfactory training for laboratory workers at a sub-professional level and as such is experimental. Its success will depend in a large measure on the continued interest, support, and guidance of official medical and hospital organizations."

The program is now in its fourth year, and 42 students have received certificates of completion of the course. In examining the distribution of employment of the aides (tabulated below) it is of interest to note that 13 of the aides are employed at the same hospital with which they affiliated during training and that seven students in the 1955-56 class who were trained in hospitals outside of Minneapolis and St. Paul are also employed in rural Minnesota.

Employment as of September, 1956, 42 Laboratory Aides

	Doctor's Office or Clinic	Hospital	Research Laboratory	Total
In Minneapolis-St. Paul	9	14	1	24
In other Minnesota Cities	1	8		9
Outside Minnesota		3		3
Married and not working				1
Unknown				5
				42

From reports and surveys of employers and graduates, it appears that the original objectives of the experiment are being realized to a satisfactory extent. With careful selection of students, it is possible in 12 months to teach a few basic laboratory techniques, limited in scope, in such a way that the students can learn these procedures well and can do these tests competently. The laboratory aides are employed in situations where they are able to make a real contribution to laboratory service by doing time-consuming "routine" tests, thereby allowing time for the medical technologist to perform more exacting and demanding procedures. Furthermore the return of the students to their community hospitals for practical training will tend to encourage employment of them in the areas where the personnel situation is most critical. The initial experimental period of 5 years will be completed in September, 1958. With present indications of satisfaction with the program it appears that this course merits continuing if funds become available to support it.

The increasing demand for laboratory personnel has resulted in a frequent suggestion that the answer is more "technicians" quickly trained in one specific area. The Registry of Medical Technologists (ASCP) does certify histologic technicians with requirements of high school graduation with one year of training

under a pathologist and one year of experience in tissue preparation. In the area of tissue work utilization of these technicians answers the specific need in a practical and satisfactory way. Likewise the laboratory aide program described previously is an attempt to provide technical help in those situations where the needs can properly be met in terms of personnel with limited training. This program is proving valuable and useful.

There continues to be, however, alarming agitation for "blood bank technicians," "hematology technicians," "cytology technicians," etc. In large hospitals with departmentalized laboratory services where supervision is available such a plan is a tempting solution to some of the pressures of work load and insufficient numbers of graduate technologists available for employment. The facts found in the Arbogast[8] survey deserve sober consideration. Sixty percent of the registered hospitals in the United States are under 100 bed capacity. The medical technologist in the laboratory of a hospital of this size generally has direct responsibility (often with only cursory medical supervision) for all the laboratory work done and must be able to do all types of laboratory tests. Seventy-one percent of the registered technologists in all sizes of hospitals perform all types of laboratory work, and only 29 percent work in fixed assignments. In view of these facts it is hardly reasonable to dispute the fact that teaching programs still have a direct responsibility to meet the greatest need; namely, for technologists well trained in fundamental principles and practices in all clinical laboratory areas.

There remains much to be done in improving the curricula in pre-technical programs and the caliber of clinical technical training. The importance of accurate laboratory work by well trained personnel cannot be minimized in the present day situation where the physician no longer performs his own tests but relies on the work of the medical technologist. Dean Diehl[9] has commented in a recent editorial upon the responsibility of organized medicine in this aspect of high standards for laboratory training and practice as follows:

> "The day seems to be passing in which the physician performs much of his own laboratory work. Important as it may be to the diagnosis and management of disease, he will expect others to perform this role. He will come to rely increasingly on the skill of these other hands. This becomes clear when one considers the large contributions that chemistry, bacteriology, hematology, and other laboratory sciences are making to the advance of American Medicine in this century.
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> "A physician is by nature a skeptical person. A report from the laboratory may be to him just a slip of paper of questionable value. Still he must come to trust the skills of laboratory workers in order that these skills may help him make the decisions he must take. For this reason, if for no other, each physician and all physicians must be vitally concerned with the establishment of high standards of laboratory training and practice.
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> "There are now many workers poorly trained; there are many

schools that exploit heartlessly the needs for workers in this field. Glowing advertisements promise a short pleasure course, immediate vocational success. At the same time, approved schools of medical technology suffer progressive decrements in enrollment. Perhaps this field needs a sober study such as the one which culminated in the Flexner report on medical education in the early part of this century. After that report, organized medicine developed and enforced high standards of medical education, closing the "diploma mills." Today, medical technology, an emerging profession under the aegis of organized medicine, needs its strenuous support and encouragement. Under the best of circumstances, it will gradually fill the need for professional clinical laboratory workers."

In this regard the results of the Belk and Sunderman[10] survey might well be reviewed. This study was conducted under well controlled conditions to check on the accuracy of some of the common chemical measurements in clinical laboratories. Fifty-nine laboratories in the Pennsylvania-New Jersey area received several samples for analyses for hemoglobin, glucose, chloride, total protein, albumin, calcium, and urea nitrogen. The results of the determinations were graded as satisfactory or unsatisfactory based on values for the various tests selected by the referee as being within the limits that should be maintained for satisfactory laboratory practice. The survey findings are tabulated below:

Substance Tested	Number of Determinations Classed Satisfactory	Number of Determinations Classed Unsatisfactory
Hemoglobin	38	65
Glucose	60	43
Sodium chloride	54	63
Total protein	18	29
Albumin	9	35
Calcium	28	59
Urea nitrogen	35	48

The authors stated, "The scatter of measurements and the degree of unreliability is surprising. The accuracy of measurements is below any reasonable standard. It will be noted that unsatisfactory results outnumbered the satisfactory and that no laboratory had a perfect score."

In a follow-up survey, the clinical pathologists in that area were asked to give their opinions as to the causes for the inferior laboratory work indicated by the survey. They believed the major contributing factor to be inadequate training of technicians and secondly insufficient numbers of technical personnel to do the amount of work requested of the laboratories.

Efforts toward better educational programs and certification of personnel in clinical laboratories have been made, particularly in recent years, by medical technologists and clinical pathologists through the Board of Schools and the Board of Registry of the American Society of Clinical Pathologists.

The Board of Schools, composed of three medical technologists

and six pathologists, assists in the inspection of schools and maintenance of educational standards and acts in an advisory capacity to the Council on Medical Education and Hospitals of the American Medical Association. The Council in 1936, with the establishment of minimum standards for clinical laboratories for the training of medical technologists, initiated approval of schools for this training. At that time 96 schools qualified for approval. In May, 1955, there were 596 approved schools with a student capacity of 4503 and an enrollment of 2790 students, or 62 percent of capacity.[6] Three hundred and six hospital schools are affiliated with teaching institutions that grant degrees in medical technology after completion of combined requirements of academic courses and clinical laboratory training.[11] The approved schools for medical technology in Minnesota include St. Luke's Hospital and St. Mary's Hospital in Duluth, Minneapolis General Hospital, Northwestern Hospital, St. Mary's Hospital, and Swedish Hospital in Minneapolis, St. Cloud Hospital in St. Cloud, and Ancker Hospital, Charles T. Miller Hospital, and St. Joseph's Hospital in St. Paul.

The Board of Registry, composed of three medical technologists and six pathologists, was established in 1928 to develop minimum standards for the training and certification of proficiency of clinical laboratory personnel. Since this time the registry has been recognized by the professional medical and hospital organizations as the only authoritative certifying body for medical technologists although there are other so-called registries in the United States. In the period from 1928 to June, 1956, the Board of Registry has issued certificates of registration to 29,320 medical technologists of which 22,894 remain currently active. Renewal of certification is required each year.

The certification of MT (ASCP) is awarded to the person who qualifies both by satisfactory completion of written examination and by education and training. At the present time the educational requirements include two years of college (90 quarter credits) of which 18 quarter hours must be in biologic science and 14 quarter hours in chemistry. The clinical training requirements include 12 consecutive months of training in all phases of medical technology in a clinical laboratory approved for training. Realizing the fact that more comprehensive and better preparation of laboratory personnel is essential with the expanding needs brought about by the growth of medical and scientific knowledge, the Board of Registry has increased the pre-technical educational requirements for certification, effective January, 1962, to 3 years of college with additional academic requirements in the basic sciences.

Medical technologists are not licensed by the State of Minnesota. Only three states (California, Florida, and Alabama) have

a requirement of licensure for people working in clinical laboratories in those states. Time does not permit here a detailed discussion of the disadvantages or merits of licensure in this field.

Medical technology is young in comparison with the well-established programs and standards in medicine and nursing. Only within the last few years has there been developing nationally a consciousness of our professional responsibility for standardization and development of educational programs. In this connection, the University of Minnesota, long a leader in medical technology education, will continue to be active in this growth not only at an undergraduate level but also at a graduate level. To meet demands for better schools and teaching programs paralleling the growth of medical knowledge and use of clinical laboratory for the care of patients, supplemental programs for the graduate in technical specialties, in teaching, and in administration must be offered.

The importance of supplemental programs in this field has been recognized for many years by the Department of Continuation Medical Education and the Center for Continuation Study. The Medical Technology staff has participated actively in planning and presenting annual courses with the cooperation of the Center for several years. There has always been a gratifying response to the courses (within the limits of the facilities of the Center) by technologists from Minnesota and the neighboring states.

A new activity, the counseling program in medical technology, although influencing only in an indirect way the standards of the Course, perhaps should be briefly mentioned in this report. Within the last decade, in cooperation with the Junior College Office of the College of Science, Literature, and the Arts, a program of counseling students in orientation and registration procedures has been slowly developing. In this way data have been collected pertaining to the abilities of our students and graduates. One potential aspect of the activity is the use of these data in developing a testing program for better selection of students. At the present time the only criterion for selection of students for this course is that of scholastic achievement. Of concern to us is the large numbers of students who start as freshmen in the Course and then cancel, primarily for poor scholarship or change of interest during the first two years. Students who reach the fourth year level generally complete the requirements for graduation. The only exception to this in recent years was in 1954 when three seniors did not graduate for reasons of poor scholastic records. The following table, based on data for the past 8 years, shows the percentage of students cancelling out of the Course in Medical Technology as com-

pared with percentages in the College of Science, Literature, and the Arts.

	Percent of Student Drop Out in Medical Technology	In SLA	
		Men	Women
From 1st to 2nd year	59	18	15
Fron 2nd to 3rd year	43	30	39
From 3rd to 4th year	23	31	27

Research on testing programs for students in this field is needed and has potential value and use not only here but also in other schools.

In conclusion, the past years have witnessed the growth and development of educational programs in a new profession of medical technology. The coming years give promise of progress toward improvement and standardization of training programs and emerging of teachers and leaders in this field. No better words can be found to express the objectives toward which the efforts of the staff are directed in continuing to prepare young men and women for this profession than those written by Dr. Beard[12] 35 years ago: "A profession is first of all of a trained body of (men). Its initial test is culture. And culture is a product of education. Its second distinction is that of social privilege, bestowed upon it in consideration of the peculiar office it has to fill. The test it carries with it is fitness. And fitness is the flower of education. Its third distinction is that of social obligation. Its ultimate test is service. And service is the function of (him) who has the capacity and will to serve."

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